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(54) Title: POTASSIUM CHANNEL INTERACTORS AND USES THEREFOR

(57) Abstract: The invention provides isolated nucleic acids molecules, designated PCIP nucleic acid molecules, which encode proteins that bind potassium channels and modulate potassium channel mediated activities. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing PCIP nucleic acid molecules, host cells into which the expression vectors have been introduced, and nonhuman transgenic animals in which a PCIP gene has been introduced or disrupted. The invention still further provides isolated PCIP proteins, fusion proteins, antigenic peptides and anti-PCIP antibodies. Diagnostic methods utilizing compositions of the invention are also provided.

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## POTASSIUM CHANNEL INTERACTORS AND USES THEREFOR

### 5 Related Applications

This application claims priority to U.S. provisional Application No. 60/110,033, filed on November 25, 1998, U.S. provisional Application No. 60/109,333, filed on November 20, 1998, U.S. provisional Application No. 60/110,277, filed on November 30, 1998, U.S. Patent Application No.: 09/298,731, filed on April 23, 1999, U.S. Patent Application No.: 09/350,614, filed on July 9, 1999, U.S. Patent Application No.: 09/350,874, filed on July 9, 1999, U.S. Patent Application No.: 09/399,913, filed on September 21, 1999, U.S. Patent Application No.: 09/400,492, filed on September 21, 1999, PCT Application No. PCT/US99/27428, filed on November 19, 1999, U.S. Patent Application No.: 09/670,756, filed on September 27, 2000, and U.S. Patent Application No.: 09/703,094 filed on 31 October 2000 incorporated herein in their entirety by this reference.

### Background of the Invention

Mammalian cell membranes are important to the structural integrity and activity of many cells and tissues. Of particular interest in membrane physiology is the study of trans-membrane ion channels which act to directly control a variety of pharmacological, physiological, and cellular processes. Numerous ion channels have been identified including calcium, sodium, and potassium channels, each of which have been investigated to determine their roles in vertebrate and insect cells.

Because of their involvement in maintaining normal cellular homeostasis, much attention has been given to potassium channels. A number of these potassium channels open in response to changes in the cell membrane potential. Many voltage-gated potassium channels have been identified and characterized by their electrophysiological and pharmacological properties. Potassium currents are more diverse than sodium or calcium currents and are further involved in determining the response of a cell to external stimuli. The diversity of potassium channels and their important physiological role highlights their potential as targets for developing therapeutic agents for various diseases.

One of the best characterized classes of potassium channels are the voltage-gated potassium channels. The prototypical member of this class is the protein encoded by the Shaker gene in *Drosophila melanogaster*. Proteins of the Shal or Kv4 family are a type of voltage-gated potassium channels that underlies many of the native A type currents that have been recorded from different primary cells. Kv4 channels have a major role in the repolarization of cardiac action potentials. In neurons, Kv4 channels and the A currents they may comprise play an important role in modulation of firing rate, action potential initiation and in controlling dendritic responses to synaptic inputs.

The fundamental function of a neuron is to receive, conduct, and transmit signals. Despite the varied purpose of the signals carried by different classes of neurons, the form of the signal is always the same and consists of changes in the electrical potential across the plasma membrane of the neuron. The plasma membrane of a neuron contains voltage-gated cation channels, which are responsible for propagating this electrical potential (also referred to as an action potential or nerve impulse) across and along the plasma membrane.

The Kv family of channels includes, among others: (1) the delayed-rectifier potassium channels, which repolarize the membrane after each action potential to prepare the cell to fire again; and (2) the rapidly inactivating (A-type) potassium channels, which are active predominantly at subthreshold voltages and act to reduce the rate at which excitable cells reach firing threshold. In addition to being critical for action potential conduction, Kv channels also control the response to depolarizing, e.g., synaptic, inputs and play a role in neurotransmitter release. As a result of these activities, voltage-gated potassium channels are key regulators of neuronal excitability (Hille B., *Ionic Channels of Excitable Membranes*, Second Edition, Sunderland, MA: Sinauer, (1992)).

There is tremendous structural and functional diversity within the Kv potassium channel superfamily. This diversity is generated both by the existence of multiple genes and by alternative splicing of RNA transcripts produced from the same gene. Nonetheless, the amino acid sequences of the known Kv potassium channels show high similarity. All appear to be comprised of four, pore forming  $\alpha$ -subunits and some are known to have four cytoplasmic ( $\beta$ -subunit) polypeptides (Jan L.Y. *et al.* (1990) *Trends Neurosci* 13:415-419, and Pongs, O. *et al.* (1995) *Sem Neurosci* 7:137-146). The

known Kv channel ( $\alpha$ -subunits fall into four sub-families named for their homology to channels first isolated from *Drosophila*: the Kv1, or *Shaker*-related subfamily; the Kv2, or *Shab*-related subfamily; the Kv3, or *Shaw*-related subfamily; and the Kv4, or *Shal*-related subfamily.

5           Kv4.2 and Kv4.3 are examples of Kv channel ( $\alpha$ -subunits of the *Shal*-related subfamily. Kv4.3 has a unique neuroanatomical distribution in that its mRNA is highly expressed in brainstem monoaminergic and forebrain cholinergic neurons, where it is involved in the release of the neurotransmitters dopamine, norepinephrine, serotonin, and acetylcholine.

10           This channel is also highly expressed in cortical pyramidal cells and in interneurons. (Serdio P. *et al.* (1996) *J. Neurophys* 75:2174-2179). Interestingly, the Kv4.3 polypeptide is highly expressed in neurons which express the corresponding mRNA. The Kv4.3 polypeptide is expressed in the somatodendritic membranes of these cells, where it is thought to contribute to the rapidly inactivating K<sup>+</sup> conductance.

15           Kv4.2 mRNA is widely expressed in brain, and the corresponding polypeptide also appears to be concentrated in somatodendritic membranes where it also contributes to the rapidly inactivating K<sup>+</sup> conductance (Sheng *et al.* (1992) *Neuron* 9:271-84). These somatodendritic A-type Kv channels, like Kv4.2 and Kv4.3, are likely involved in processes which underlie learning and memory, such as integration of sub-threshold  
20           synaptic responses and the conductance of back-propagating action potentials (Hoffman D.A. *et al.* (1997) *Nature* 387:869-875).

          Thus, proteins which interact with and modulate the activity of potassium channel proteins *e.g.*, potassium channels having a Kv4.2 or Kv4.3 subunit, provide novel molecular targets to modulate neuronal or cardiac excitability, *e.g.*, action  
25           potential conduction, somatodendritic excitability and neurotransmitter release, in cells expressing these channels. In addition, detection of genetic lesions in the gene encoding these proteins could be used to diagnose and treat central nervous system disorders such as epilepsy, spinocerebellar ataxia, anxiety, depression, age-related memory loss, migraine, obesity, Parkinsons disease or Alzheimer's disease; or cardiovascular disorders  
30           such as heart failure, hypertension, atrial fibrillation, dilated cardiomyopathy, idiopathic cardiomyopathy, or angina.



### **Summary of the Invention**

The present invention is based, at least in part, on the discovery of novel nucleic acid molecules which encode gene products that interact with potassium channel proteins or possess substantial homology to the gene products of the invention that  
5 interact with potassium channel proteins (paralogs). Potassium channel proteins are, for example, potassium channels having a Kv4.2 or Kv4.3 subunit. The nucleic acid molecules of the invention and their gene products are referred to herein as "Potassium Channel Interacting Proteins", "PCIP", or "KChIP" nucleic acid and protein molecules. The PCIP proteins of the present invention interact with, *e.g.*, bind to a potassium  
10 channel protein, modulate the activity of a potassium channel protein, and/or modulate a potassium channel mediated activity in a cell, *e.g.*, a neuronal or cardiac cell. The PCIP molecules of the present invention are useful as modulating agents to regulate a variety of cellular processes, *e.g.*, neuronal or cardiac cell processes. Accordingly, in one aspect, this invention provides isolated nucleic acid molecules encoding PCIP proteins  
15 or biologically active portions thereof, as well as nucleic acid fragments suitable as primers or hybridization probes for the detection of PCIP-encoding nucleic acids.

In one embodiment, a PCIP nucleic acid molecule of the invention is at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more identical to the nucleotide sequence (*e.g.*, to the entire length of the nucleotide sequence) shown in SEQ  
20 ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID  
25 NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as  
30 Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, or a complement thereof.

In another preferred embodiment, the isolated nucleic acid molecule includes the nucleotide sequence shown SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102, or a complement thereof. In another preferred embodiment, the nucleic acid molecule includes a fragment of at least 300, 350, 400, 426, 471, or 583 nucleotides of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102, or a complement thereof.

In another embodiment, a PCIP nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence sufficiently identical to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID

NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994. In a preferred embodiment, a PCIP nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more identical to the amino acid sequence of SEQ ID NO: 2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994.

In another preferred embodiment, an isolated nucleic acid molecule encodes the amino acid sequence of 1v, 9q, p19, W28559, KChIP4a, KChIP4b, 33b07, 1p, and rat 7s protein. In yet another preferred embodiment, the nucleic acid molecule includes a nucleotide sequence encoding a protein having the amino acid sequence of SEQ ID NO: 2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109, or the amino acid sequence encoded by the DNA insert of

the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994. In yet another preferred embodiment, the nucleic acid molecule is at least 426, 471, or 583 nucleotides in length and encodes a protein having

5 a PCIP activity (as described herein).

Another embodiment of the invention features nucleic acid molecules, preferably PCIP nucleic acid molecules, which specifically detect PCIP nucleic acid molecules relative to nucleic acid molecules encoding non-PCIP proteins. For example, in one embodiment, such a nucleic acid molecule is at least 426, 400-450, 471, 450-500, 500-

10 550, 583, 550-600, 600-650, 650-700, 700-750, 750-800 or more nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3 SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ

15 ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID

20 NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, or a complement thereof. In preferred embodiments, the

25 nucleic acid molecules are at least 15 (*e.g.*, contiguous) nucleotides in length and hybridize under stringent conditions to nucleotides 93-126, 360-462, 732-825, 1028-1054, or 1517-1534 of SEQ ID NO:7. In other preferred embodiments, the nucleic acid molecules comprise nucleotides 93-126, 360-462, 732-825, 1028-1054, or 1517-1534 of SEQ ID NO:7.

30 In other preferred embodiments, the nucleic acid molecules are at least 15 (*e.g.*, contiguous) nucleotides in length and hybridize under stringent conditions to nucleotides 1-14, 49-116, 137-311, 345-410, 430-482, 503-518, 662-693, 1406-1421, 1441-1457,

1478-1494, or 1882-1959 of SEQ ID NO:13. In other preferred embodiments, the nucleic acid molecules comprise nucleotides 1-14, 49-116, 137-311, 345-410, 430-482, 503-518, 662-693, 1406-1421, 1441-1457, 1478-1494, or 1882-1959 of SEQ ID NO:13.

In preferred embodiments, the nucleic acid molecules are at least 15 (*e.g.*,  
 5 contiguous) nucleotides in length and hybridize under stringent conditions to nucleotides 932-1527, 1548-1765, 1786-1871, 1908-2091, 2259-2265, or 2630-2654 of SEQ ID NO:35. In other preferred embodiments, the nucleic acid molecules comprise nucleotides 932-1527, 1548-1765, 1786-1871, 1908-2091, 2259-2265, or 2630-2654 of SEQ ID NO:35.

10 In other preferred embodiments, the nucleic acid molecule encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID  
 15 NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID  
 20 NO:103, or SEQ ID NO:109 or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3 SEQ ID NO:5, SEQ ID  
 25 NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID  
 30 NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID

NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102 under stringent conditions.

Another embodiment of the invention provides an isolated nucleic acid molecule which is antisense to a PCIP nucleic acid molecule, *e.g.*, the coding strand of a PCIP  
5 nucleic acid molecule.

Another aspect of the invention provides a vector comprising a PCIP nucleic acid molecule. In certain embodiments, the vector is a recombinant expression vector. In another embodiment, the invention provides a host cell containing a vector of the invention. The invention also provides a method for producing a protein, preferably a  
10 PCIP protein, by culturing in a suitable medium, a host cell, *e.g.*, a mammalian host cell such as a non-human mammalian cell, of the invention containing a recombinant expression vector, such that the protein is produced.

Another aspect of this invention features isolated or recombinant PCIP proteins and polypeptides. In one embodiment, the isolated protein, preferably a PCIP protein,  
15 includes at least one calcium binding domain. In a preferred embodiment, the protein, preferably a PCIP protein, includes at least one calcium binding domain and has an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more identical to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID  
20 NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID  
25 NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951,  
30 98991, 98993, or 98994. In another preferred embodiment, the protein, preferably a PCIP protein, includes at least one calcium binding domain and modulates a potassium channel mediated activity. In yet another preferred embodiment, the protein, preferably

a PCIP protein, includes at least one calcium binding domain and is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102.

In another embodiment, the invention features fragments of the proteins having the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109, wherein the fragment comprises at least 15 amino acids (*e.g.*, contiguous amino acids) of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID

NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with the ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994. In another  
5 embodiment, the protein, preferably a PCIP protein, has the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID  
10 NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109.

15 In another embodiment, the invention features an isolated protein, preferably a PCIP protein, which is encoded by a nucleic acid molecule having a nucleotide sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more identical to a nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID  
20 NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID  
25 NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102, or a complement thereof.

The proteins of the present invention or biologically active portions thereof, can be operatively linked to a non-PCIP polypeptide (*e.g.*, heterologous amino acid  
30 sequences) to form fusion proteins. The invention further features antibodies, such as monoclonal or polyclonal antibodies, that specifically bind proteins of the invention, preferably PCIP proteins. In addition, the PCIP proteins or biologically active portions



thereof can be incorporated into pharmaceutical compositions, which optionally include pharmaceutically acceptable carriers.

In another aspect, the present invention provides a method for detecting the presence of a PCIP nucleic acid molecule, protein or polypeptide in a biological sample  
5 by contacting the biological sample with an agent capable of detecting a PCIP nucleic acid molecule, protein or polypeptide such that the presence of a PCIP nucleic acid molecule, protein or polypeptide is detected in the biological sample.

In another aspect, the present invention provides a method for detecting the presence of PCIP activity in a biological sample by contacting the biological sample  
10 with an agent capable of detecting an indicator of PCIP activity such that the presence of PCIP activity is detected in the biological sample.

In another aspect, the invention provides a method for modulating PCIP activity comprising contacting a cell capable of expressing PCIP with an agent that modulates PCIP activity such that PCIP activity in the cell is modulated. In one embodiment, the  
15 agent inhibits PCIP activity. In another embodiment, the agent stimulates PCIP activity. In one embodiment, the agent is an antibody that specifically binds to a PCIP protein. In another embodiment, the agent modulates expression of PCIP by modulating transcription of a PCIP gene or translation of a PCIP mRNA. In yet another embodiment, the agent is a nucleic acid molecule having a nucleotide sequence that is  
20 antisense to the coding strand of a PCIP mRNA or a PCIP gene.

In one embodiment, the methods of the present invention are used to treat a subject having a disorder characterized by aberrant PCIP protein or nucleic acid expression or activity by administering an agent which is a PCIP modulator to the subject. In one embodiment, the PCIP modulator is a PCIP protein. In another  
25 embodiment the PCIP modulator is a PCIP nucleic acid molecule. In yet another embodiment, the PCIP modulator is a peptide, peptidomimetic, or other small molecule. In a preferred embodiment, the disorder characterized by aberrant PCIP protein or nucleic acid expression is a CNS disorder or a cardiovascular disorder.

The present invention also provides a diagnostic assay for identifying the  
30 presence or absence of a genetic alteration characterized by at least one of (i) aberrant modification or mutation of a gene encoding a PCIP protein; (ii) mis-regulation of the

gene; and (iii) aberrant post-translational modification of a PCIP protein, wherein a wild-type form of the gene encodes a protein with a PCIP activity.

In another aspect the invention provides a method for identifying a compound that binds to or modulates the activity of a PCIP protein, by providing an indicator composition comprising a PCIP protein having PCIP activity, contacting the indicator composition with a test compound, and determining the effect of the test compound on PCIP activity in the indicator composition to identify a compound that modulates the activity of a PCIP protein.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

#### **Brief Description of the Drawings**

*Figure 1* depicts the cDNA sequence and predicted amino acid sequence of human 1v. The nucleotide sequence corresponds to nucleic acids 1 to 1463 of SEQ ID NO:1. The amino acid sequence corresponds to amino acids 1 to 216 of SEQ ID NO:2.

*Figure 2* depicts the cDNA sequence and predicted amino acid sequence of rat 1v. The nucleotide sequence corresponds to nucleic acids 1 to 1856 of SEQ ID NO:3. The amino acid sequence corresponds to amino acids 1 to 245 of SEQ ID NO:4.

*Figure 3* depicts the cDNA sequence and predicted amino acid sequence of mouse 1v. The nucleotide sequence corresponds to nucleic acids 1 to 1907 of SEQ ID NO:5. The amino acid sequence corresponds to amino acids 1 to 216 of SEQ ID NO:6.

*Figure 4* depicts the cDNA sequence and predicted amino acid sequence of rat 1vl. The nucleotide sequence corresponds to nucleic acids 1 to 1534 of SEQ ID NO:7. The amino acid sequence corresponds to amino acids 1 to 227 of SEQ ID NO:8.

*Figure 5* depicts the cDNA sequence and predicted amino acid sequence of mouse 1vl. The nucleotide sequence corresponds to nucleic acids 1 to 1540 of SEQ ID NO:9. The amino acid sequence corresponds to amino acids 1 to 227 of SEQ ID NO:10.

*Figure 6* depicts the cDNA sequence and predicted amino acid sequence of the partial rat 1vn. The nucleotide sequence corresponds to nucleic acids 1 to 955 of SEQ ID NO:11. The amino acid sequence corresponds to amino acids 1 to 203 of SEQ ID NO:12. (The full length rat 1vn sequences are set forth herein in Figure 63, see below).

*Figure 7* depicts the cDNA sequence and predicted amino acid sequence of human 9ql. The nucleotide sequence corresponds to nucleic acids 1 to 2009 of SEQ ID NO:13. The amino acid sequence corresponds to amino acids 1 to 270 of SEQ ID NO:14.

5        *Figure 8* depicts the cDNA sequence and predicted amino acid sequence of rat 9ql. The nucleotide sequence corresponds to nucleic acids 1 to 1247 of SEQ ID NO:15. The amino acid sequence corresponds to amino acids 1 to 257 of SEQ ID NO:16.

10       *Figure 9* depicts the cDNA sequence and predicted amino acid sequence of mouse 9ql. The nucleotide sequence corresponds to nucleic acids 1 to 2343 of SEQ ID NO:17. The amino acid sequence corresponds to amino acids 1 to 270 of SEQ ID NO:18.

15       *Figure 10* depicts the cDNA sequence and predicted amino acid sequence of human 9qm. The nucleotide sequence corresponds to nucleic acids 1 to 1955 of SEQ ID NO:19. The amino acid sequence corresponds to amino acids 1 to 252 of SEQ ID NO:20.

*Figure 11* depicts the cDNA sequence and predicted amino acid sequence of rat 9qm. The nucleotide sequence corresponds to nucleic acids 1 to 2300 of SEQ ID NO:21. The amino acid sequence corresponds to amino acids 1 to 252 of SEQ ID NO:22.

20       *Figure 12* depicts the cDNA sequence and predicted amino acid sequence of human 9qs. The nucleotide sequence corresponds to nucleic acids 1 to 1859 of SEQ ID NO:23. The amino acid sequence corresponds to amino acids 1 to 220 of SEQ ID NO:24.

25       *Figure 13* depicts the cDNA sequence and predicted amino acid sequence of monkey 9qs. The nucleotide sequence corresponds to nucleic acids 1 to 2191 of SEQ ID NO:25. The amino acid sequence corresponds to amino acids 1 to 220 of SEQ ID NO:26.

30       *Figure 14* depicts the cDNA sequence and predicted amino acid sequence of rat 9qc. The nucleotide sequence corresponds to nucleic acids 1 to 2057 of SEQ ID NO:27. The amino acid sequence corresponds to amino acids 1 to 252 of SEQ ID NO:28.

*Figure 15* depicts the cDNA sequence and predicted amino acid sequence of rat 8t. The nucleotide sequence corresponds to nucleic acids 1 to 1904 of SEQ ID NO:29. The amino acid sequence corresponds to amino acids 1 to 225 of SEQ ID NO:30.

5 *Figure 16* depicts the cDNA sequence and predicted amino acid sequence of human p19. The nucleotide sequence corresponds to nucleic acids 1 to 619 of SEQ ID NO:31. The amino acid sequence corresponds to amino acids 1 to 200 of SEQ ID NO:32.

10 *Figure 17* depicts the cDNA sequence and predicted amino acid sequence of rat p19. The nucleotide sequence corresponds to nucleic acids 1 to 442 of SEQ ID NO:33. The amino acid sequence corresponds to amino acids 1 to 109 of SEQ ID NO:34.

*Figure 18* depicts the cDNA sequence and predicted amino acid sequence of mouse p19. The nucleotide sequence corresponds to nucleic acids 1 to 2644 of SEQ ID NO:35. The amino acid sequence corresponds to amino acids 1 to 256 of SEQ ID NO:36.

15 *Figure 19* depicts the cDNA sequence and predicted amino acid sequence of human W28559. The nucleotide sequence corresponds to nucleic acids 1 to 380 of SEQ ID NO:37. The amino acid sequence corresponds to amino acids 1 to 126 of SEQ ID NO:38.

20 *Figure 20* depicts the cDNA sequence and predicted amino acid sequence of human P193. The nucleotide sequence corresponds to nucleic acids 1 to 2176 of SEQ ID NO:39. The amino acid sequence corresponds to amino acids 1 to 41 of SEQ ID NO:40.

*Figure 21* depicts a schematic representation of the rat 1v, the rat 9qm, and the mouse P19 proteins, aligned to indicate the conserved domains among these proteins.

25 *Figure 22* depicts the genomic DNA sequence of human 9q. *Figure 22A* depicts exon 1 and its flanking intron sequences (SEQ ID NO:46). *Figure 22B* depicts exons 2-11 and the flanking intron sequences (SEQ ID NO:47).

*Figure 23* depicts the cDNA sequence and predicted amino acid sequence of monkey KChIP4a. The nucleotide sequence corresponds to nucleic acids 1 to 2413 of SEQ ID NO:48. The amino acid sequence corresponds to amino acids 1 to 233 of SEQ ID NO:49.

*Figure 24* depicts the cDNA sequence and predicted amino acid sequence of monkey KChIP4b. The nucleotide sequence corresponds to nucleic acids 1 to 1591 of SEQ ID NO:50. The amino acid sequence corresponds to amino acids 1 to 233 of SEQ ID NO:51.

5        *Figure 25* depicts an alignment of KChIP4a, KChIP4b, 9ql, 1v, p19, and related human paralog (hsncspara) W28559. Amino acids identical to the consensus are shaded in black, conserved amino acids are shaded in gray.

*Figure 26* depicts the cDNA sequence and predicted amino acid sequence of rat 33b07. The nucleotide sequence corresponds to nucleic acids 1 to 2051 of SEQ ID  
10 NO:52. The amino acid sequence corresponds to amino acids 1 to 407 of SEQ ID NO:53.

*Figure 27* depicts the cDNA sequence and predicted amino acid sequence of human 33b07. The nucleotide sequence corresponds to nucleic acids 1 to 4148 of SEQ ID NO:54. The amino acid sequence corresponds to amino acids 1 to 414 of SEQ ID  
15 NO:55.

*Figure 28* depicts the cDNA sequence and predicted amino acid sequence of rat 1p. The nucleotide sequence corresponds to nucleic acids 1 to 2643 of SEQ ID NO:56. The amino acid sequence corresponds to amino acids 1 to 267 of SEQ ID NO:57.

*Figure 29* depicts the cDNA sequence and predicted amino acid sequence of rat  
20 7s. The nucleotide sequence corresponds to nucleic acids 1 to 2929 of SEQ ID NO:58. The amino acid sequence corresponds to amino acids 1 to 270 of SEQ ID NO:59.

*Figure 30* depicts the cDNA sequence and predicted amino acid sequence of rat 29x. The nucleotide sequence corresponds to nucleic acids 1 to 1489 of SEQ ID NO:60. The amino acid sequence corresponds to amino acids 1 to 351 of SEQ ID NO:61.

25        *Figure 31* depicts the cDNA sequence of rat 25r. The nucleotide sequence corresponds to nucleic acids 1 to 1194 of SEQ ID NO:62.

*Figure 32* depicts the cDNA sequence and predicted amino acid sequence of rat 5p. The nucleotide sequence corresponds to nucleic acids 1 to 600 of SEQ ID NO:63. The amino acid sequence corresponds to amino acids 1 to 95 of SEQ ID NO:64.

30        *Figure 33* depicts the cDNA sequence and predicted amino acid sequence of rat 7q. The nucleotide sequence corresponds to nucleic acids 1 to 639 of SEQ ID NO:65. The amino acid sequence corresponds to amino acids 1 to 212 of SEQ ID NO:66.

*Figure 34* depicts the cDNA sequence and predicted amino acid sequence of rat 19r. The nucleotide sequence corresponds to nucleic acids 1 to 816 of SEQ ID NO:67. The amino acid sequence corresponds to amino acids 1 to 271 of SEQ ID NO:68.

*Figure 35* depicts the cDNA sequence and predicted amino acid sequence of  
5 monkey KChIP4c. The nucleotide sequence corresponds to nucleic acids 1 to 2263 of  
SEQ ID NO:69. The amino acid sequence corresponds to amino acids 1 to 229 of SEQ  
ID NO:70.

*Figure 36* depicts the cDNA sequence and predicted amino acid sequence of  
monkey KChIP4d. The nucleotide sequence corresponds to nucleic acids 1 to 2259 of  
10 SEQ ID NO:71. The amino acid sequence corresponds to amino acids 1 to 250 of SEQ  
ID NO:72.

*Figure 37* depicts an alignment of KChIP4a, KChIP4b, KChIP4c, and KChIP4d.

*Figure 38* depicts a graph showing the current traces from CHO cells which  
express Kv4.2 with or without KChIP2 (9ql). Cells are voltage clamped at -80 mV and  
15 stepped from -60 mV to +50 mV for 200ms. Peak current amplitudes at the various test  
voltages are shown in the right panel. *Figure 38* further depicts a table showing the  
amplitude and kinetic effects of KChIP2 (9ql) on Kv4.2. KChIP2 expression alters the  
peak current amplitude, inactivation and recovery from inactivation time constants, and  
activation  $V_{1/2}$ .

*Figure 39* depicts a graph showing the current traces from CHO cells which  
express Kv4.2 with or without KChIP3 (p19). Cells are voltage clamped at -80 mV and  
stepped from -60 mV to +50 mV for 200ms. Peak current amplitudes at the various test  
voltages are shown in the right panel. *Figure 39* further depicts a table showing the  
amplitude and kinetic effects of KChIP3 (p19) on Kv4.2. KChIP3 causes alterations in  
25 peak current and inactivation and recovery from inactivation time constants.

*Figure 40* depicts results from electrophysiological experiments demonstrating  
that coexpression of KChIP1 dramatically alters the current density and kinetics of  
Kv4.2 channels expressed in CHO cells.

*Figure 40A* depicts current traces from a Kv4.2 transfected CHO cell. Current  
30 was evoked by depolarizing the cell sequentially from a holding potential of -80 mV to  
test potentials from -60 to 50 mV. Current traces are leak subtracted using a p/5  
protocol. The current axis is shown at the same magnification as in (b) to emphasize the

change in current amplitudes. Inset- Single current trace at 50mV at an expanded current axis to show the kinetics of current activation and inactivation.

*Figure 40B* depicts current traces as in (a), but from a cell transfected with equal amounts of DNA for Kv4.2 and KChIP1.

5 *Figure 40C* depicts peak current amplitude at all voltages from cells transfected with Kv4.2 alone (n=11) or cotransfected with KChIP1 (n=9).

*Figures 40D and 40E* depict recovery from inactivation using a two pulse protocol. Kv4.2 alone (D) or coexpressed with KChIP1 (E) is driven into the inactivated state using a first pulse to 50 mV, then a second pulse to 50 mV is applied at varying  
10 times after the first pulse. Holding potential is -80 mV before and after all pulses.

*Figure 40F* depicts a summary of the percentage the peak current recovers between pulses for Kv4.2 (n=8) and Kv4.2 plus KChIP1 (n=5) transfected cells. The time constant of recovery from inactivation is fit to a single exponential.

*Figure 41* depicts an alignment of human KChIP family members with closely  
15 related members of the recoverin family of Ca<sup>2+</sup> sensing proteins. (HIP:human hippocalcin; NCS1:rat neuronal calcium sensor 1). The alignment was performed using the MegAlign program for Macintosh (version 4.00 from DNASTAR) using the Clustal method with the PAM250 residue weight table and default parameters, and shaded using BOXSHADES. Residues identical to the consensus are shaded black, conservative  
20 substitutions are shaded grey. X, Y, Z and -X, -Y, -Z denote the positions of residues which are responsible for binding to the calcium ion in the EF hand.

*Figure 42* depicts a physical map of the IOSCA region.

*Figure 43* depicts a linkage map showing the location of h9q and known markers associating with IOSCA and epilepsy.

25 *Figure 44* depicts the cDNA sequence and predicted amino acid sequence of human 1vl (KChIP1l). The nucleotide sequence corresponds to nucleic acids 1 to 1477 of SEQ ID NO:79. The alternation of upper and lower case letters indicates the individual exons. The KChIP1l (KChIP1long) specific exon is the second exon in the indicated sequence. The amino acid sequence corresponds to amino acids 1 to 227 of  
30 SEQ ID NO:109.

*Figure 45* depicts the cDNA sequence and predicted amino acid sequence of an N-terminal splice variant of human KChIP1N. The nucleotide sequence corresponds to nucleic acids 1 to 1639 of SEQ ID NO:80. The amino acid sequence corresponds to amino acids 1 to 232 of SEQ ID NO:81.

*Figure 46* depicts an alignment of the N-terminal domains of the rat and human KChIP1N, indicating that this N-terminal domain is conserved between the two sequences.

*Figure 47* depicts the genomic DNA sequence of human KChIP2 (including  
 5 KChIP2 l, m, s, and N). The nucleotide sequence corresponds to nucleic acids 1 to 17,803 of SEQ ID NO:74. Upper case letters indicate the exons and lower case letters indicate the introns.

*Figure 48* depicts the cDNA sequence and predicted amino acid sequence of the rat KChIP2L. The nucleotide sequence corresponds to nucleic acids 1 to 1285 of SEQ  
 10 ID NO:75. The amino acid sequence corresponds to amino acids 1 to 270 of SEQ ID NO:76.

*Figure 49* depicts the cDNA sequence and predicted amino acid sequence of the human 8t (KChIP2N). The nucleotide sequence corresponds to nucleic acids 1 to 2076 of SEQ ID NO:77. The amino acid sequence corresponds to amino acids 1 to 225 of  
 15 SEQ ID NO:78.

*Figure 50* depicts an alignment of the N-terminal domains of the rat and human KChIP2N (8t) proteins, indicating that these proteins exhibit a 96.5% identity.

*Figure 51* depicts the cDNA sequence and predicted amino acid sequence of the full length human KChIP3. The nucleotide sequence corresponds to nucleic acids 1 to  
 20 2835 of SEQ ID NO:82. The amino acid sequence corresponds to amino acids 1 to 256 of SEQ ID NO:83. The alternation of upper and lower case letters indicates the individual exons.

*Figure 52* depicts the cDNA sequence and predicted amino acid sequence of the rat KChIP3. The nucleotide sequence corresponds to nucleic acids 1 to 2414 of SEQ ID  
 25 NO:84. The amino acid sequence corresponds to amino acids 1 to 178 of SEQ ID NO:85. Upper case letters indicate the coding region and lower case letters indicate the 3' UTR.

*Figure 53* depicts the cDNA sequence and predicted amino acid sequence of the monkey KChIP4XC (KChIP4b). The nucleotide sequence corresponds to nucleic acids  
 30 1 to 1005 of SEQ ID NO:86. The amino acid sequence corresponds to amino acids 1 to 127 of SEQ ID NO:87.

*Figure 54* depicts the cDNA sequence and predicted amino acid sequence of the mouse KChIP4N2 (KChIP4c). The nucleotide sequence corresponds to nucleic acids 1 to 2181 of SEQ ID NO:88. The amino acid sequence corresponds to amino acids 1 to  
 35 229 of SEQ ID NO:89.

*Figure 55* depicts the cDNA sequence and predicted amino acid sequence of the rat KChIP4. The nucleotide sequence corresponds to nucleic acids 1 to 2022 of SEQ ID



NO:90. The amino acid sequence corresponds to amino acids 1 to 198 of SEQ ID NO:91.

*Figure 56* depicts the cDNA sequence and predicted amino acid sequence of the human KChIP4aS (KChIP4N1S) a shorter splice variant of KChIP4N1. The nucleotide sequence corresponds to nucleic acids 1 to 2366 of SEQ ID NO:92. The amino acid sequence corresponds to amino acids 1 to 188 of SEQ ID NO:93.

*Figure 57* depicts the cDNA sequence and predicted amino acid sequence of the human KChIP4a (KChIP4N1). The nucleotide sequence corresponds to nucleic acids 1 to 2431 of SEQ ID NO:94. The amino acid sequence corresponds to amino acids 1 to 233 of SEQ ID NO:95.

*Figure 58* depicts the cDNA sequence and predicted amino acid sequence of the human KChIP4c (KChIPN2). The nucleotide sequence corresponds to nucleic acids 1 to 2261 of SEQ ID NO:96. The amino acid sequence corresponds to amino acids 1 to 229 of SEQ ID NO:97.

*Figure 59* depicts the cDNA sequence and predicted amino acid sequence of the human KChIP4d (KChIP4N3). The nucleotide sequence corresponds to nucleic acids 1 to 2299 of SEQ ID NO:98. The amino acid sequence corresponds to amino acids 1 to 250 of SEQ ID NO:99.

*Figure 60* depicts the cDNA sequence and predicted amino acid sequence of the rat KChIP4N1x, a splice variant of KChIP4N1. The nucleotide sequence corresponds to nucleic acids 1 to 2246 of SEQ ID NO:100. The amino acid sequence corresponds to amino acids 1 to 272 of SEQ ID NO:101.

*Figure 61* is a set of graphs depicting the competitive modulation of Kv4.3 inactivation time constant by KChIP4N2 and KChIP1. The cRNA species injected are listed in the cRNA section with 4.3 indicating Kv4.3, 1 indicating KChIP1, and 4 indicating KChIP4. The numbers in the parentheses indicate dilution factors of cRNA injected with 1x=stock solution. The triangles above the bar graphs illustrate combination of fixed amount of KChIP4N2 or KChIP1 and increasing amount of KChIP1 or KChIP4N2, respectively.

*Figure 62* depicts protein alignments indicating that the N-terminal domains of human KChIP1N and monkey KChIP4N2 are homologous and that the N-terminal domains of human/rat KChIP1 and monkey KChIP4N2 are divergent.

*Figure 63* depicts the cDNA sequence and predicted amino acid sequence of the rat KChIP1N (1vn). The nucleotide sequence corresponds to nucleic acids 1 to 1856 of SEQ ID NO:102. The amino acid sequence corresponds to amino acids 1 to 232 of SEQ ID NO:103.

*Figure 64* is a graph depicting the concentration-dependent modulation of Kv4.3 and Kv4.3/KChIP1 currents in *Xenopus* oocytes by arachidonic acid. Depolarizing

pulses from a holding potential of  $-80$  mV to  $+40$  mV (duration = 500 ms). Arachidonic acid at  $1$ - $10$   $\mu$ M inhibited peak amplitudes (A) and decreased inactivation time constants ( $\tau_{\text{inact}}$ ) (B) in oocytes injected with Kv4.3 cRNA itself (solid line) and those co-injected with both Kv4.3 and KChIP1 cRNA (dashed line).  $n = 5$  oocytes for each data point.

5 *Figure 65* is a graph depicting the modulation of Kv4.3 and Kv4.3/KChIP1 currents by arachidonic acid is reversible. Currents in *Xenopus* oocytes were evoked every 7 seconds with depolarizing pulses to  $+40$  mV (duration = 500 ms) from a holding potential of  $-80$  mV. Effects on peak amplitude (A) and inactivation time constants ( $\tau_{\text{inact}}$ ) (B) are shown with shaded bars indicating application of  $10$   $\mu$ M arachidonic acid  
10 and open bars wash-out with ND96 medium supplemented with  $0.5$  mg/ml BSA ( $n = 5$  for each data point).

*Figure 66* is a graph depicting the modulation of Kv4.3 and Kv4.3/KChIP1 by fatty acids. (A) Percentage block of Kv4 (open bars) and Kv4.3/KChIP (shaded bars) peak amplitudes by  $10$   $\mu$ M linolelaidic acid ( $n = 9, 8$ , Kv4.3, Kv4.3/KChIP1,  
15 respectively),  $\gamma$ -linolenic acid ( $n = 9, 8$ ), ETI ( $n = 4, 6$ ), ETYA ( $n = 4, 6$ ), and arachidonic acid ( $n = 8, 9$ ) in *Xenopus* oocytes. All values except that of linolelaidic acid/Kv4.3 alone were statistically significant when compared to no fatty acid controls. Differences of all values between Kv4.3 and Kv4.3+KChIP1 for all fatty acids were statistically insignificant. (B) Percentage inhibition of inactivation time constants ( $\tau_{\text{inact}}$ )  
20 of currents in panel A under the same conditions. Values are presented as mean  $\pm$  SEM. All values for Kv4.3 alone were not statistically significant compared to no-fatty acid control. All values for Kv4.3+KChIP1 except that of linolelaidic acid were statistically significant compared to no-fatty acid control. The differences of values between Kv4.3 and Kv4.3+KChIP1 within every fatty acid treatment except linolelaidic acid were  
25 significant.

*Figure 67* is a graph indicating that Arachidonic acid does not interfere with association between KChIP1 and the N-terminal domain of Kv4.3. (A) Superimposed sensograms showing that neither the association phase nor the dissociation phase of interaction between the intracellular N-terminal domain of Kv4.3 and KChIP1 was  
30 qualitatively changed by  $10$   $\mu$ M arachidonic acid in Biosensor assays. (B) N-terminal domain of Kv4.3 and KChIP1 interaction-dependent growth in selective SC-WLH medium was not altered by  $10$   $\mu$ M of ETYA. The non-selective medium SC-WL, which allowed strains to grow independently of the interaction between the N-terminal domain of Kv4.3 and KChIP1, was used to control the non-specific effects of ETYA on growth  
35 of the strains. Values are presented as mean  $\pm$  SEM.  $n = 4$  for each data point.

*Figure 68* is a graph depicting the results from a Taqman analysis of rat KChIP1N tissue expression.

### **Detailed Description of the Invention**

The present invention is based, at least in part, on the discovery of novel nucleic acid molecules which encode gene products that interact with potassium channel proteins or possess substantial homology to the gene products of the invention that interact with potassium channel proteins (paralogs). Potassium channel proteins are, for example, potassium channels having a Kv4.2 or Kv4.3 subunit. The nucleic acid molecules of the invention and their gene products are referred to herein as "Potassium Channel Interacting Proteins", "PCIP", or "KChIP" nucleic acid and protein molecules. Preferably, the PCIP proteins of the present invention interact with, *e.g.*, bind to a potassium channel protein, modulate the activity of a potassium channel protein, and/or modulate a potassium channel mediated activity in a cell, *e.g.*, a neuronal or cardiac cell.

As used herein, the term "PCIP family" when referring to the protein and nucleic acid molecules of the invention is intended to mean two or more proteins or nucleic acid molecules having a PCIP activity as defined herein. Such PCIP family members can be naturally or non-naturally occurring and can be from either the same or different species. For example, a PCIP family can contain a first protein of human origin, as well as other, distinct proteins of human origin or alternatively, can contain homologues of non-human origin.

As used interchangeably herein, a "PCIP activity", "biological activity of PCIP" or "functional activity of PCIP", refers to an activity exerted by a PCIP protein, polypeptide or nucleic acid molecule on a PCIP responsive cell or on a PCIP protein substrate, as determined *in vivo*, or *in vitro*, according to standard techniques. In one embodiment, a PCIP activity is a direct activity, such as an association with a PCIP-target molecule. As used herein, a "target molecule" or "binding partner" is a molecule with which a PCIP protein binds or interacts in nature, such that PCIP-mediated function is achieved. A PCIP target molecule can be a non-PCIP molecule or a PCIP protein or polypeptide of the present invention. In an exemplary embodiment, a PCIP target molecule is a PCIP ligand. Alternatively, a PCIP activity is an indirect activity, such as a cellular signaling activity mediated by interaction of the PCIP protein with a PCIP ligand. The biological activities of PCIP are described herein.

For example, the PCIP proteins of the present invention can have one or more of the following activities: (1) they can interact with (*e.g.*, bind to) a potassium channel

protein or portion thereof; (2) they can regulate the phosphorylation state of a potassium channel protein or portion thereof; (3) they can associate with (*e.g.*, bind) calcium and can, for example, act as calcium dependent kinases, *e.g.*, phosphorylate a potassium channel or a G-protein coupled receptor in a calcium-dependent manner; (4) they can  
 5 associate with (*e.g.*, bind) calcium and can, for example, act in a calcium-dependent manner in cellular processes, *e.g.*, act as calcium dependent transcription factors; (5) they can modulate a potassium channel mediated activity in a cell (*e.g.*, a neuronal cell such as a sensory neuron cell or a motor neuron cell, or a cardiac cell) to, for example, beneficially affect the cell; (6) they can modulate chromatin formation in a cell, *e.g.*, a  
 10 neuronal or cardiac cell; (7) they can modulate vesicular traffic and protein transport in a cell, *e.g.*, a neuronal or cardiac cell; (8) they can modulate cytokine signaling in a cell, *e.g.*, a neuronal or cardiac cell; (9) they can regulate the association of a potassium channel protein or portion thereof with the cellular cytoskeleton; (10) they can modulate cellular proliferation; (11) they can modulate the release of neurotransmitters; (12) they  
 15 can modulate membrane excitability; (13) they can influence the resting potential of membranes; (14) they can modulate wave forms and frequencies of action potentials; and (15) they can modulate thresholds of excitation.

As used herein, a "potassium channel" includes a protein or polypeptide that is involved in receiving, conducting, and transmitting signals in an excitable cell.

20 Potassium channels are typically expressed in electrically excitable cells, *e.g.*, neurons, cardiac, skeletal and smooth muscle, renal, endocrine, and egg cells, and can form heteromultimeric structures, *e.g.*, composed of pore-forming and cytoplasmic subunits. Examples of potassium channels include: (1) the voltage-gated potassium channels, (2) the ligand-gated potassium channels, and (3) the mechanically-gated potassium  
 25 channels. For a detailed description of potassium channels, see Kandel E.R. *et al.*, Principles of Neural Science, second edition, (Elsevier Science Publishing Co., Inc., N.Y. (1985)), the contents of which are incorporated herein by reference. The PCIP proteins of the present invention have been shown to interact with, for example, potassium channels having a Kv4.3 subunit or a Kv4.2 subunit.

30 As used herein, a "potassium channel mediated activity" includes an activity which involves a potassium channel, *e.g.*, a potassium channel in a neuronal cell or a cardiac cell, associated with receiving, conducting, and transmitting signals in, for

example, the nervous system or in the heart. Potassium channel mediated activities include release of neurotransmitters, *e.g.*, dopamine or norepinephrine, from cells, *e.g.*, neuronal or cardiac cells; modulation of resting potential of membranes, wave forms and frequencies of action potentials, and thresholds of excitation; and modulation of processes such as integration of sub-threshold synaptic responses and the conductance of back-propagating action potentials in, for example, neuronal cells or cardiac cells.

As the PCIP proteins of the present invention modulate potassium channel mediated activities, they may be useful as novel diagnostic and therapeutic agents for potassium channel associated disorders and/or nervous system related disorders.

Moreover, the PCIP proteins of the present invention modulate Kv4 potassium channels, *e.g.*, potassium channels having a Kv4.2 or Kv4.3 subunit, which underlie the voltage-gated K<sup>+</sup> current known as I<sub>to</sub> (transient outward current) in the mammalian heart (Kaab S. *et al.* (1998) *Circulation* 98(14):1383-93; Dixon J.E. *et al.* (1996) *Circulation Research* 79(4):659-68; Nerbonne JM (1998) *Journal of Neurobiology* 37(1):37-59; Barry D.M. *et al.* (1998) *Circulation Research* 83(5):560-7; Barry D.M. *et al.* (1996) *Annual Review of Physiology* 58:363-94. This current underlies the rapid repolarization of cardiac myocytes during an action potential. It also participates in the inter-beat interval by controlling the rate at which cardiac myocytes reach the threshold for firing a subsequent action potential.

This current is also known to be down regulated in patients with cardiac hypertrophy, resulting in prolongation of the cardiac action potential. In these patients, action potential prolongation is thought to produce changes in calcium load and calcium handling within the myocardium, which contributes to the progression of cardiac disease from hypertrophy to heart failure (Wickenden *et al.* (1998) *Cardiovascular Research* 37:312). Interestingly, several PCIPs of the present invention (*e.g.*, 9ql, 9qm, 9qs, shown in SEQ ID NOs:13, 15, 17, 19, 21, 23, and 25) bind to and modulate potassium channels containing a Kv4.2 or Kv4.3 subunit and contain calcium binding EF-hand domains. Because of mutations in these PCIP genes, defects in the expression of these calcium-binding PCIP proteins themselves, or defects in the interaction between these PCIPs and Kv4.2 or Kv4.3 channels, might be expected to lead to decreases in KV4.3 or Kv4.3(I<sub>m</sub>) currents in the myocardium, therapeutic agents that alter PCIP expression or modulate the interaction between these PCIPs and Kv4.2 or Kv4.3 may be extremely

valuable agents to slow or prevent the progression of disease from hypertrophy to heart failure.

As used herein, a "potassium channel associated disorder" includes a disorder, disease or condition which is characterized by a misregulation of a potassium channel mediated activity. Potassium channel associated disorders can detrimentally affect conveyance of sensory impulses from the periphery to the brain and/or conductance of motor impulses from the brain to the periphery; integration of reflexes; interpretation of sensory impulses; and emotional, intellectual (*e.g.*, learning and memory), or motor processes. Potassium channel associated disorders can further detrimentally affect electrical impulses that stimulate the cardiac muscle fibers to contract. Examples of potassium channel associated disorders include nervous system related disorders, as well as cardiovascular disorders.

As used herein, a "nervous system related disorder" includes a disorder, disease or condition which affects the nervous system. Examples of potassium channel associated disorders and nervous system related disorders include cognitive disorders, *e.g.*, memory and learning disorders, such as amnesia, apraxia, agnosia, amnesic dysnomia, amnesic spatial disorientation, Kluver-Bucy syndrome, Alzheimer's related memory loss (Eglen R.M. (1996) *Pharmacol. and Toxicol.* 78(2):59-68; Perry E.K. (1995) *Brain and Cognition* 28(3):240-58) and learning disability; disorders affecting consciousness, *e.g.*, visual hallucinations, perceptual disturbances, or delirium associated with Lewy body dementia; schitzo-effective disorders (Dean B. (1996) *Mol. Psychiatry* 1(1):54-8), schizophrenia with mood swings (Bymaster F.P. (1997) *J. Clin. Psychiatry* 58 (suppl.10):28-36; Yeomans J.S. (1995) *Neuropharmacol.* 12(1):3-16; Reimann D. (1994) *J. Psychiatric Res.* 28(3):195-210), depressive illness (primary or secondary); affective disorders (Janowsky D.S. (1994) *Am. J. Med. Genetics* 54(4):335-44); sleep disorders (Kimura F. (1997) *J. Neurophysiol.* 77(2):709-16), *e.g.*, REM sleep abnormalities in patients suffering from, for example, depression (Riemann D. (1994) *J. Psychosomatic Res.* 38 Suppl. 1:15-25; Bourgin P. (1995) *Neuroreport* 6(3): 532-6), paradoxical sleep abnormalities (Sakai K. (1997) *Eur. J. Neuroscience* 9(3):415-23), sleep-wakefulness, and body temperature or respiratory depression abnormalities during sleep (Shuman S.L. (1995) *Am. J. Physiol.* 269(2 Pt 2):R308-17; Mallick B.N. (1997) *Brain Res.* 750(1-2):311-7). Other examples of nervous system related disorders include

disorders affecting pain generation mechanisms, *e.g.*, pain related to irritable bowel syndrome (Mitch C.H. (1997) *J. Med. Chem.* 40(4):538-46; Shannon H.E. (1997) *J. Pharmac. and Exp. Therapeutics* 281(2):884-94; Bouaziz H. (1995) *Anesthesia and Analgesia* 80(6):1140-4; or Guimaraes A.P. (1994) *Brain Res.* 647(2):220-30) or chest  
5 pain; movement disorders (Monassi C.R. (1997) *Physiol. and Behav.* 62(1):53-9), *e.g.*, Parkinson's disease related movement disorders (Finn M. (1997) *Pharmacol. Biochem. & Behavior* 57(1-2):243-9; Mayorga A.J. (1997) *Pharmacol. Biochem. & Behavior* 56(2):273-9); eating disorders, *e.g.*, insulin hypersecretion related obesity (Maccario M. (1997) *J. Endocrinol. Invest.* 20(1):8-12; Premawardhana L.D. (1994) *Clin. Endocrinol.*  
10 40(5): 617-21); drinking disorders, *e.g.*, diabetic polydipsia (Murzi E. (1997) *Brain Res.* 752(1-2):184-8; Yang X. (1994) *Pharmacol. Biochem. & Behavior* 49(1):1-6); neurodegenerative disorders, *e.g.*, Alzheimer's disease, dementias related to Alzheimer's disease (such as Pick's disease), Parkinson's and other Lewy diffuse body diseases, multiple sclerosis, amyotrophic lateral sclerosis, progressive supranuclear palsy,  
15 epilepsy, spinocerebellar ataxia, epileptic syndromes, and Jakob-Creutzfeldt disease; psychiatric disorders, *e.g.*, depression, schizophrenic disorders, Korsakoff's psychosis, mania, anxiety disorders, bipolar affective disorders, or phobic disorders; neurological disorders, *e.g.*, migraine; spinal cord injury; stroke; and head trauma.

As used herein, "epilepsy" includes a common neurological disorder caused by  
20 disturbances in the normal electrical functions of the brain. In normal brain function millions of tiny electrical charges pass from nerve cells in the brain to all parts of the body. In patients with epilepsy, this normal pattern is interrupted by sudden and unusually intense bursts of electrical energy, which may briefly affect a person's consciousness, bodily movements, or sensations. These physical changes are called  
25 epileptic seizures. There are two categories of seizures: partial seizures, which occur in one area of the brain, and generalized seizures, which affect nerve cells throughout the brain. Epilepsy may result from a brain injury before, during, or after birth; head trauma; poor nutrition; some infectious diseases; brain tumors; and some poisons. However, in many cases the cause is unknown. Attacks of epilepsy may be preceded by  
30 a feeling of unease or sensory discomfort called an aura, which indicates the beginning of the seizure. Signs of an impending epileptic seizure, which vary among patients, may include visual phenomena such as flickering lights or "sunbursts." Recently, a genetic

linkage for epilepsy has been found on chromosome 10q, near marker D10S192: 10q22-q24 (Ottman *et al.* (1995) *Nature Genetics* 10:56-60). The many forms of epilepsy include: grand mal, Jacksonian, myoclonic progressive familial, petit mal, Lennox-Gastaut syndrome, febrile seizures, psycho-motor, and temporal lobe. The observations  
5 described herein are particularly useful in developing treatments for partial epilepsy.

As used herein, "ataxia" includes a common neurological disorder caused by disturbances in the normal electrical functions of the brain. Spinocerebellar ataxia type 1 (SCA1) is an autosomal dominant disorder which is genetically linked to the short arm of chromosome 6 based on linkage to the human major histocompatibility complex  
10 (HLA). See, for example, H. Yakura *et al.* (1974) *N. Engl. J. Med.*, 291, 154-155; and J. F. Jackson *et al.* (1977) *N. Engl. J. Med* 296, 1138-1141. SCA1 has been shown to be tightly linked to the marker D6S89 on the short arm of chromosome 6, telomeric to HLA. See, for example, L. P. W. Ranum *et al.*, *Am. J. Hum. Genet.*, 49, 31-41 (1991); and H. Y. Zoghbi *et al.*, *Am. J. Hum. Genet.*, 49, 23-30 (1991). The  
15 observations described herein are particularly useful in developing treatments for infantile onset spinocerebellar ataxia (IOSCA).

As used herein, a "cardiovascular disorder" includes a disorder affecting the cardiovascular system, *e.g.*, the heart. Examples of cardiovascular disorders include arteriosclerosis, ischemia reperfusion injury, restenosis, arterial inflammation, vascular  
20 wall remodeling, ventricular remodeling, rapid ventricular pacing, coronary microembolism, tachycardia, bradycardia, pressure overload, aortic bending, coronary artery ligation, vascular heart disease, atrial fibrillation, long-QT syndrome, congestive heart failure, sinus node dysfunction, angina, heart failure, hypertension, atrial fibrillation, atrial flutter, dilated cardiomyopathy, idiopathic cardiomyopathy,  
25 myocardial infarction, coronary artery disease, coronary artery spasm, or arrhythmia. In a preferred embodiment, the cardiovascular disorder is associated with an abnormal  $I_{to}$  current.

Some members of a PCIP family may also have common structural characteristics, such as a common structural domain or motif or a sufficient amino acid  
30 or nucleotide sequence homology as defined herein. Such PCIP family members can be naturally or non-naturally occurring and can be from either the same or different species. For example, a PCIP family can contain a first protein of human origin, as well as other,



distinct proteins of human origin or alternatively, can contain homologues of non-human origin.

For example, members of a PCIP family which have common structural characteristics, may comprise at least one "calcium binding domain". As used herein,  
 5 the term "calcium binding domain" includes an amino acid domain, *e.g.*, an EF hand (Baimbridge K.G. *et al.* (1992) *TINS* 15(8): 303-308), which is involved in calcium binding. Preferably, a calcium binding domain has a sequence, which is substantially identical to the consensus sequence:

10 EO••OO••ODKDGDG•O•••EF••OO. (SEQ ID NO:41).

O can be I, L, V or M, and "•" indicates a position with no strongly preferred residue. Each residue listed is present in more than 25% of sequences, and those underlined are present in more than 80% of sequences. Amino acid residues 126-154 and 174-202 of  
 15 the human 1v protein, amino acid residues 126-154 and 174-202 of the rat 1v protein, amino acid residues 137-165 and 185-213 of the rat 1vl protein, amino acid residues 142-170 of the rat 1vn protein, amino acid residues 126-154 and 174-202 of the mouse 1v protein, amino acid residues 137-165 and 185-213 of the mouse 1vl protein, amino acid residues 144-172, 180-208, and 228-256 of the human 9q1 protein, amino acid  
 20 residues 126-154, 162-190, and 210-238 of the human 9qm protein, amino acid residues 94-122, 130-158, and 178-206 of the human 9qs protein, amino acid residues 126-154, 162-190, and 210-238 of the rat 9qm protein, amino acid residues 131-159, 167-195, and 215-243 of the rat 9ql protein, amino acid residues 126-154, 162-190, and 210-238 of the rat 9qc protein, amino acid residues 99-127, 135-163, and 183-211 of the rat 8t  
 25 protein, amino acid residues 144-172, 180-208, and 228-256 of the mouse 9ql protein, amino acid residues 94-122, 130-158, and 178-206 of the monkey 9qs protein, amino acid residues 94-122, 130-158, and 178-206 of the human p19 protein, amino acid residues 19-47 and 67-95 of the rat p19 protein, and amino acid residues 130-158, 166-194, and 214-242 of the mouse p19 protein comprise calcium binding domains (EF  
 30 hands) (see Figure 21). Amino acid residues 116-127 and 152-163 of the monkey KChIP4a and KChIP4b proteins comprise calcium binding domains.

In another embodiment, the isolated PCIP proteins of the present invention are identified based on the presence of at least one conserved carboxyl-terminal domain which includes an amino acid sequence of about 100-200 amino acid residues in length, preferably 150-200 amino acid residues in length, and more preferably 185 amino acid residues in length, and which includes three EF hands. PCIP proteins of the present invention preferably contain a carboxyl-terminal domain which is at least about 70%, 71%, 74%, 75%, 76%, 80%, or more identical to the carboxyl terminal 185 amino acid residues of rat 1v, rat 9q, or mouse p19 (see Figures 21, 25, and 41).

Members of the PCIP family which also have common structural characteristics are listed in Table I. Other members of the PCIP family, *e.g.*, members of the PCIP family which do not have common structural characteristics, are listed in Table II and are described below. The present invention provides a full length human and a partial length rat 33b07 clone and the proteins encoded by these cDNAs. The present invention further provides partial length rat 1p clone and the protein encoded by this cDNA. In addition, the present invention provides a partial length rat 7s clone and the protein encoded by this cDNA.

The present invention further provides PCIP family members which represent previously identified cDNAs (29x, 25r, 5p, 7q, and 19r). These previously identified cDNAs are identified herein as PCIP family members, *i.e.*, as molecules which have a PCIP activity, as described herein. Accordingly, the present invention provides methods for using these previously identified cDNAs, *e.g.*, methods for using these cDNAs in the screening assays, the diagnostic assays, the prognostic assays, and the methods of treatment described herein.

The PCIP molecules of the present invention were initially identified based on their ability, as determined using yeast two-hybrid assays (described in detail in Example 1), to interact with the amino-terminal 180 amino acids of rat Kv4.3 subunit. Further binding studies with other potassium subunits were performed to demonstrate specificity of the PCIP for Kv4.3 and Kv4.2. *In situ* localization, immuno-histochemical methods, co-immunoprecipitation and patch clamping methods were then used to clearly demonstrate that the PCIPs of the present invention interact with and modulate the activity of potassium channels, particularly those comprising a 4.3 or 4.2 subunit.

Several novel human, mouse, monkey, and rat PCIP family members have been identified, referred to herein as 1v, 9q, p19, W28559, KChIP4, 33b07, 1p, and rat 7s proteins and nucleic acid molecules. The human, rat, and mouse cDNAs encoding the 1v polypeptide are represented by SEQ ID NOs:1, 3, and 5, and shown in Figures 1, 2, and 3, respectively. In the brain, 1v mRNA is highly expressed in neocortical and hippocampal interneurons, in the thalamic reticular nucleus and medial habenula, in basal forebrain and striatal cholinergic neurons, in the superior colliculus, and in cerebellar granule cells. The 1v polypeptide is highly expressed in the somata, dendrites, axons and axon terminals of cells that express 1v mRNA. Splice variants of the 1v gene have been identified in rat and mouse and are represented by SEQ ID NOs: 7, 9, and 11 and shown in Figures 4, 5, and 6, respectively. 1v polypeptide interacts with potassium channels comprising Kv4.3 or kv4.2 subunits, but not with Kv1.1 subunits. As determined by Northern blot, the 1v transcripts (mRNA) are expressed predominantly in the brain

The 8t cDNA (SEQ ID NO: 29) encodes a polypeptide having a molecular weight of approximately 26 kD corresponding to SEQ ID NO:30 (see Figure 15). The 8t polypeptide interacts with potassium channel comprising Kv4.3 or Kv4.2 subunits, but not with Kv1.1 subunits. As determined by Northern blot and *in situ* data, the 8t mRNA is expressed predominantly in the heart and the brain. The 8t cDNA is a splice variant of 9q.

Human, rat, monkey, and mouse 9q cDNA were also isolated. Splice variants include human 9ql (SEQ ID NO:13; Figure 7) rat 9ql (SEQ ID NO:15; Figure 8), mouse 9ql (SEQ ID NO:17; Figure 9), human 9qm (SEQ ID NO:19; Figure 10), rat 9qm (SEQ ID NO:21; Figure 11), human 9qs (SEQ ID NO:23; Figure 12), monkey 9qs (SEQ ID NO:25; Figure 13), and rat 9qc (SEQ ID NO:27; Figure 14). The genomic DNA sequence of 9q has also be determined. Exon 1 and its flanking intron sequences (SEQ ID NO:46) are shown in Figure 22A. Exons 2-11 and the flanking intron sequences (SEQ ID NO:47) are shown in Figure 22B. 9q polypeptides interact with potassium channels comprising Kv4.3 or Kv4.2 subunits, but not with Kv1.1 subunits. As determined by Northern blot and *in situ* data, the 9q proteins are expressed predominantly in the heart and the brain. In the brain, 9q mRNA is highly expressed in

the neostriatum, hippocampal formation, neocortical pyramidal cells and interneurons, and in the thalamus, superior colliculus, and cerebellum.

Human, rat, and mouse P19 cDNA was also isolated. Human P19 is shown in SEQ ID NO:31 and Figure 16; and in SEQ ID NO:39 and Figure 20 (the 3' sequence).

5 Rat P19 is shown in SEQ ID NO:33 and Figure 17, and mouse P19 is shown in SEQ ID NO:35 and Figure 18. P19 polypeptides interact with potassium channels comprising Kv4.3 or Kv4.2 subunits, but not with Kv1.1 subunits. As determined by Northern blot analysis, the P19 transcripts (mRNA) are expressed predominantly in the brain.

A partial human paralog of the PCIP molecules was also identified. This paralog  
10 is referred to herein as W28559 and is shown in SEQ ID NO:37 and Figure 19.

Monkey KChIP4a and its splice variants KChIP4b, KChIP4c, and KChIP4d were also identified. Monkey KChIP4a is shown in SEQ ID NO:48 and Figure 23. Monkey KChIP4b is shown in SEQ ID NO:50 and Figure 24. Monkey KChIP4c is shown in  
15 SEQ ID NO:69 and Figure 35. Monkey KChIP4d is shown in SEQ ID NO:71 and Figure 36.

The nucleotide sequence of the full length rat 33b07 cDNA and the predicted amino acid sequence of the rat 33b07 polypeptide are shown in Figure 26 and in SEQ ID NOs:52 and 53, respectively. The rat 33b07 cDNA encodes a protein having a  
20 molecular weight of approximately 44.7 kD and which is 407 amino acid residues in length. Rat 33b07 binds rKv4.3N and rKv4.2N with slight preference for rKv4.2N in yeast 2-hybrid assays.

The nucleotide sequence of the full length human 33b07 cDNA and the predicted amino acid sequence of the human 33b07 polypeptide are shown in Figure 27 and in  
SEQ ID NOs:54 and 55, respectively.

25 The nucleotide sequence of the partial length rat 1p cDNA and the predicted amino acid sequence of the rat 1p polypeptide are shown in Figure 28 and in SEQ ID NOs:56 and 57, respectively. The rat 1p cDNA encodes a protein having a molecular weight of approximately 28.6 kD and which is 267 amino acid residues in length. Rat 1p binds rKv4.3N and rKv4.2N with slight preference for rKv4.3N in yeast two-hybrid  
30 assays.

The nucleotide sequence of the partial length rat 7s cDNA and the predicted amino acid sequence of the rat 7s polypeptide are shown in Figure 29 and in SEQ ID

NOs:58 and 59, respectively. The rat 7s cDNA encodes a protein having a molecular weight of approximately 28.6 kD and which is 270 amino acid residues in length. Rat 7s binds rKv4.3N and rKv4.2N with preference for rKv4.3N in yeast two-hybrid assays.

5 The sequences of the present invention are summarized below, in Tables I and II.

**Table I**

Novel Polynucleotides and Polypeptides of the Present Invention (full length except where noted)

PCIP	Nucleic Acid Molecule Form	Source	SEQ ID NO: DNA	SEQ ID NO: PROTEIN	ATCC
1v or KChIP1	1v	human (225-875)*	1	2	98994
	KChIP1N (1vN) N-terminal splice variant	human (353-461)	80	81	
	1v	rat (210-860)	3	4	98946
	1v	mouse (477-1127)	5	6	98945
	1vl	human	79	109	
	1vl	rat (31-714)	7	8	98942
	1vl	Mouse (77-760)	9	10	98943
	1vn	rat (345-955)  (339-1037)	11 (partial)  102 (full)	12 (partial)  103 (full)	98944

9q or KChIP2	Genomic DNA sequence	human	74		
	Genomic DNA sequence (Exon 1 and flanking intron sequences)	human	46		
	Genomic DNA sequence (Exons 2-11 and flanking intron sequences)	human	47		
	9ql	human (207-1019)	13	14	98993 98991
	9ql	rat (2-775)  (1-813)	15 (partial)  75 (full length)	16 (partial)  76 (full length)	98948
	9ql	mouse (181 -993)	17	18	98937
	9qm	human (207-965)	19	20	98993 98991
	9qm	rat (214-972)	21	22	98941
	9qs	human (207-869)	23	24	98951
	9qs	monkey (133-795)	25	26	98950
	9qc	rat (208-966)	27	28	98947

	8t	Human (1-678)	77 (partial)	78 (partial)	
		rat (1-678)	29 (partial)	30 (partial)	98939
p19 or KChIP3	KChIP3 (full length)	Human (16-786)	82	83	
	p19	human (1-771)	31	32	PTA-316
	p19	rat (1-330)  (1-579)	33 (partial)  84 (partial)	34 (partial)  85 (partial)	98936
	p19	mouse (49-819)	35	36	98940
	p193 (partial)	Human (2-127)	39	40	98949
W28559	W28559 (partial)	human (1-339)	37	38	
KChIP4	KChIP4a (KChIP4N1)	human (248-949)	94	95	
	KChIP4aS (KChIP4N1S) shorter splice variant of KChIP4N1	human (319-885)	92	93	
	KChIP4c (KChIP4N2)	Human (90-779)	96	97	
	KChIP4d (KChIP4N3)	Human (65-817)	98	99	
	KChIP4a (KChIP4N1)	Monkey (265-966)	48	49	

	KChIP4b C-terminal splice variant	Monkey (265-966)	50 (partial)	51 (partial)	
	KChIP4b (KChIP4XC)	Monkey (1-385)	86 (partial)	87 (partial)	
	KChIP4c (KChIP4N2) splice variant	Monkey (122-811)	69	70	
	KChIP4d (KChIP4N3) splice variant	Monkey (64-816)	71	72	
	KChIP4c (KChIP4N2)	Mouse (56-745)	88	89	
	KChIP4	Rat (1-597)	90 (partial)	91 (partial)	
	KChIP4aX (KChIP4N1x) splice variant of KChIP4N1	Rat (1-821)	100 (partial)	101 (partial)	

\* The coordinates of the coding sequence are shown in parenthesis. The first column indicates the PCIPs which were identified and column 2 indicates the various nucleic acid forms identified for each PCIP.

## 5 Table II

Polynucleotides and Polypeptides of the Present Invention (full length except where noted)

PCIP	Nucleic Acid Molecule Form	Source	SEQ ID NO: DNA	SEQ ID NO: PROTEIN	ATCC
33b07 Novel	33b07	Human (88-1332)	52	53	PTA-316



	33b07	Rat (85-1308)	54	55	
1p Novel	1p (partial)	Rat (1-804)	56	57	
7s Novel	7s (partial)	Rat (1-813)	58	59	
29x	29x	Rat (433-1071)	60	61	
	25r splice variant of 29x	Rat (130-768)	62		
5p	5p	Rat (52-339)	63	64	
7q	7q	Rat (1-639)	65	66	
19r	19r	Rat (1-816)	67	68	

\* The coordinates of the coding sequence are shown in parenthesis. The first column indicates the four families of PCIPs which were identified and column 2 indicates the various nucleic acid forms identified for each family. Novel molecules are also indicated.

- 5           Plasmids containing the nucleotide sequences encoding human, rat and monkey PCIPs were deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on November 17, 1998, and assigned the Accession Numbers described above. These deposits will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of
- 10 Microorganisms for the Purposes of Patent Procedure. These deposits were made merely as a convenience for those of skill in the art and are not an admission that a deposit is required under 35 U.S.C. §112.

- Clones containing cDNA molecules encoding human p19 (clone EphP19) and human 33b07 (clone Eph33b07) were deposited with American Type Culture Collection
- 15 (Manassas, VA) on July 8, 1998 as Accession Number PTA-316, as part of a composite

deposit representing a mixture of two strains, each carrying one recombinant plasmid harboring a particular cDNA clone. (The ATCC strain designation for the mixture of hP19 and h33b07 is EphP19h33b07mix).

To distinguish the strains and isolate a strain harboring a particular cDNA clone,  
5 an aliquot of the mixture can be streaked out to single colonies on LB plates supplemented with 100 ug/ml ampicillin, single colonies grown, and then plasmid DNA extracted using a standard miniprep procedure. Next, a sample of the DNA miniprep can be digested with NotI and the resultant products resolved on a 0.8% agarose gel using standard DNA electrophoresis conditions. The digest gives the  
10 following band patterns: EphP19: 7 kb 9 (single band), Eph33b07: 5.8 kb (single band).

Various aspects of the invention are described in further detail in the following subsections:

15 I. Isolated Nucleic Acid Molecules

One aspect of the invention pertains to isolated nucleic acid molecules that encode PCIP proteins or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes to identify PCIP-encoding nucleic acid molecules (*e.g.*, PCIP mRNA) and fragments for use as PCR primers for the  
20 amplification or mutation of PCIP nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA) and RNA molecules (*e.g.*, mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

25 An "isolated" nucleic acid molecule is one which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments,  
30 the isolated PCIP nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover,

an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

- 5           A nucleic acid molecule of the present invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3 SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID
- 10 NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID
- 15 NO:100, or SEQ ID NO:102, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all
- 20 or portion of the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3 SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID
- 25 NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102, or the nucleotide sequence of the DNA
- 30 insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, as a hybridization probe, PCIP nucleic acid

molecules can be isolated using standard hybridization and cloning techniques (e.g., as described in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

5           Moreover, a nucleic acid molecule encompassing all or a portion of SEQ ID NO:1, SEQ ID NO:3 SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID  
10 NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102, or the  
15 nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994 can be isolated by the polymerase chain reaction (PCR) using synthetic oligonucleotide primers designed based upon the sequence of SEQ ID NO:1, SEQ ID NO:3 SEQ ID NO:5, SEQ  
20 ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID  
25 NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938,  
30 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994.

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis.

5 Furthermore, oligonucleotides corresponding to PCIP nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

In a preferred embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3 SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, or a portion of any of these nucleotide sequences.

In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3 SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID

NO:100, or SEQ ID NO:102, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, or a portion of any of these nucleotide sequences. A

5 nucleic acid molecule which is complementary to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3 SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID

10 NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID

15 NO:102, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, is one which is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID

20 NO:9, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3 SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID

25 NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID

30 NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102, or the

nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, thereby forming a stable duplex.

5 In still another preferred embodiment, an isolated nucleic acid molecule of the present invention comprises a nucleotide sequence which is at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more identical to the entire length of the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3 SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID  
10 NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID  
15 NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102, or the entire length of the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946,  
20 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, or a portion of any of these nucleotide sequences.

Moreover, the nucleic acid molecule of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3 SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17,  
25 SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID  
30 NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102, or the nucleotide sequence of the DNA insert of the

plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, for example a fragment which can be used as a probe or primer or a fragment encoding a biologically active portion of a PCIP protein. The

5 nucleotide sequence determined from the cloning of the PCIP gene allows for the generation of probes and primers designed for use in identifying and/or cloning other PCIP family members, as well as PCIP homologues from other species.

The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes

10 under stringent conditions to at least about 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, or 75 consecutive nucleotides of a sense sequence of SEQ ID NO:1, SEQ ID NO:3 SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID

15 NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID

20 NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, of an anti-sense sequence of SEQ ID NO:1, SEQ ID NO:3 SEQ ID NO:5, SEQ

25 ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID

30 NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID



NO:98, SEQ ID NO:100, or SEQ ID NO:102, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, or of a naturally occurring allelic variant or  
5 mutant of SEQ ID NO:1, SEQ ID NO:3 SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID  
10 NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102, or the nucleotide sequence of the DNA insert of the plasmid deposited with  
15 ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994. In an exemplary embodiment, a nucleic acid molecule of the present invention comprises a nucleotide sequence which is 350-400, 400-450, 450-500, 500-550, 550-600, 600-650, 650-700, 700-750, 750-800, 800-850, 850-900, 949, 950-1000, or more  
20 nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:1, SEQ ID NO:3 SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID  
25 NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID  
30 NO:100, or SEQ ID NO:102, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939,

98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994.

Probes based on the PCIP nucleotide sequences can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In preferred  
5 embodiments, the probe further comprises a label group attached thereto, *e.g.*, the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress a PCIP protein, such as by measuring a level of a PCIP-encoding nucleic acid in a sample of cells from a subject *e.g.*, detecting PCIP mRNA  
10 levels or determining whether a genomic PCIP gene has been mutated or deleted.

A nucleic acid fragment encoding a "biologically active portion of a PCIP protein" can be prepared by isolating a portion of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ  
15 ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID  
20 NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, which  
25 encodes a polypeptide having a PCIP biological activity (the biological activities of the PCIP proteins are described herein), expressing the encoded portion of the PCIP protein (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of the PCIP protein.

The invention further encompasses nucleic acid molecules that differ from the  
30 nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ

ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102 or the nucleotide sequence of the DNA insert of the plasmid deposited with 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, due to degeneracy of the genetic code and thus encode the same PCIP proteins as those encoded by the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102 or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID

NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109.

In addition to the PCIP nucleotide sequences shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, 5 SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID 10 NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 15 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the PCIP proteins may exist within a population (*e.g.*, the human population). Such genetic polymorphism in the PCIP genes may exist among individuals within a population due to natural allelic variation. As used herein, the terms 20 "gene" and "recombinant gene" refer to nucleic acid molecules which include an open reading frame encoding a PCIP protein, preferably a mammalian PCIP protein, and can further include non-coding regulatory sequences, and introns.

Allelic variants of human PCIP include both functional and non-functional PCIP proteins. Functional allelic variants are naturally occurring amino acid sequence 25 variants of the human PCIP protein that maintain the ability to bind a PCIP ligand and/or modulate any of the PCIP activities described herein. Functional allelic variants will typically contain only conservative substitution of one or more amino acids of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ 30 ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID

NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109 or substitution, deletion or insertion of non-critical residues

5 in non-critical regions of the protein.

Non-functional allelic variants are naturally occurring amino acid sequence variants of the human PCIP protein that do not have the ability to either bind a PCIP ligand and/or modulate any of the PCIP activities described herein. Non-functional allelic variants will typically contain a non-conservative substitution, a deletion, or

10 insertion or premature truncation of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID

15 NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109 or a substitution, insertion or deletion in critical residues or critical regions.

20 The present invention further provides non-human orthologues of the human PCIP protein. Orthologues of the human PCIP protein are proteins that are isolated from non-human organisms and possess the same PCIP ligand binding and/or modulation of potassium channel mediated activities of the human PCIP protein. Orthologues of the human PCIP protein can readily be identified as comprising an amino acid sequence that

25 is substantially identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID

30 NO:57, SEQ ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID

NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109.

Moreover, nucleic acid molecules encoding other PCIP family members and, thus, which have a nucleotide sequence which differs from the PCIP sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994 are intended to be within the scope of the invention. For example, another PCIP cDNA can be identified based on the nucleotide sequence of human PCIP. Moreover, nucleic acid molecules encoding PCIP proteins from different species, and thus which have a nucleotide sequence which differs from the PCIP sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102 or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994 are intended to be within

the scope of the invention. For example, a mouse PCIP cDNA can be identified based on the nucleotide sequence of a human PCIP.

Nucleic acid molecules corresponding to natural allelic variants and homologues of the PCIP cDNAs of the invention can be isolated based on their homology to the  
5 PCIP nucleic acids disclosed herein using the cDNAs disclosed herein, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 15, 20, 25, 30 or more nucleotides in length and hybridizes under  
10 stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID  
15 NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID  
20 NO:102 or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994. In other embodiment, the nucleic acid is at least 30, 50, 100, 150, 200, 250, 300, 307, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 949, or 950 nucleotides  
25 in length. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% identical to each other typically remain hybridized to each other. Preferably, the conditions are such that sequences at least about 70%, more preferably at least about 80%, even more preferably at least about 85% or 90% identical to each other typically  
30 remain hybridized to each other.

Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, Ausubel *et al.*, eds., John Wiley & Sons, Inc. (1995), sections 2, 4, and 6. Additional stringent conditions can be found in *Molecular Cloning: A Laboratory Manual*, Sambrook *et al.*, Cold Spring Harbor Press, Cold Spring Harbor, NY (1989), chapters 7, 9, and 11. A preferred, non-limiting example of stringent hybridization conditions includes hybridization in 4X sodium chloride/sodium citrate (SSC), at about 65-70°C (or alternatively hybridization in 4X SSC plus 50% formamide at about 42-50°C) followed by one or more washes in 1X SSC, at about 65-70°C. A preferred, non-limiting example of highly stringent hybridization conditions includes hybridization in 1X SSC, at about 65-70°C (or alternatively hybridization in 1X SSC plus 50% formamide at about 42-50°C) followed by one or more washes in 0.3X SSC, at about 65-70°C. A preferred, non-limiting example of reduced stringency hybridization conditions includes hybridization in 4X SSC, at about 50-60°C (or alternatively hybridization in 6X SSC plus 50% formamide at about 40-45°C) followed by one or more washes in 2X SSC, at about 50-60°C. Ranges intermediate to the above-recited values, *e.g.*, at 65-70°C or at 42-50°C are also intended to be encompassed by the present invention. SSPE (1xSSPE is 0.15M NaCl, 10mM NaH<sub>2</sub>PO<sub>4</sub>, and 1.25mM EDTA, pH 7.4) can be substituted for SSC (1X SSC is 0.15M NaCl and 15mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes each after hybridization is complete. The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10°C less than the melting temperature ( $T_m$ ) of the hybrid, where  $T_m$  is determined according to the following equations. For hybrids less than 18 base pairs in length,  $T_m(^{\circ}\text{C}) = 2(\# \text{ of A + T bases}) + 4(\# \text{ of G + C bases})$ . For hybrids between 18 and 49 base pairs in length,  $T_m(^{\circ}\text{C}) = 81.5 + 16.6(\log_{10}[\text{Na}^+]) + 0.41(\% \text{G+C}) - (600/\text{N})$ , where N is the number of bases in the hybrid, and  $[\text{Na}^+]$  is the concentration of sodium ions in the hybridization buffer ( $[\text{Na}^+]$  for 1X SSC = 0.165 M). It will also be recognized by the skilled practitioner that additional reagents may be added to hybridization and/or wash buffers to decrease non-specific hybridization of nucleic acid molecules to membranes, for example, nitrocellulose or nylon membranes, including but not limited to blocking agents (*e.g.*, BSA or salmon or herring sperm carrier DNA), detergents (*e.g.*, SDS),



chelating agents (*e.g.*, EDTA), Ficoll, PVP and the like. When using nylon membranes, in particular, an additional preferred, non-limiting example of stringent hybridization conditions is hybridization in 0.25-0.5M NaH<sub>2</sub>PO<sub>4</sub>, 7% SDS at about 65°C, followed by one or more washes at 0.02M NaH<sub>2</sub>PO<sub>4</sub>, 1% SDS at 65°C (see *e.g.*, Church and Gilbert  
 5 (1984) *Proc. Natl. Acad. Sci. USA* 81:1991-1995), or alternatively 0.2X SSC, 1% SDS.

Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1 corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs  
 10 in nature (*e.g.*, encodes a natural protein).

In addition to naturally-occurring allelic variants of the PCIP sequences that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID  
 15 NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID  
 20 NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946,  
 25 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, thereby leading to changes in the amino acid sequence of the encoded PCIP proteins, without altering the functional ability of the PCIP proteins. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9,  
 30 SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID

NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of PCIP (*e.g.*, the sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the PCIP proteins of the present invention, are predicted to be particularly unamenable to alteration. Furthermore, additional amino acid residues that are conserved between the PCIP proteins of the present invention and other members of the PCIP family of proteins are not likely to be amenable to alteration.

Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding PCIP proteins that contain changes in amino acid residues that are not essential for activity. Such PCIP proteins differ in amino acid sequence from SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID

NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109, yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109.

An isolated nucleic acid molecule encoding a PCIP protein homologous to the protein of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109 can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID

NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced into SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994 by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine,

tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a PCIP protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a PCIP coding sequence, such as by saturation mutagenesis, and the

5 resultant mutants can be screened for PCIP biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID  
10 NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID  
15 NO:98, SEQ ID NO:100, or SEQ ID NO:102, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

20 In a preferred embodiment, a mutant PCIP protein can be assayed for the ability to (1) interact with (*e.g.*, bind to) a potassium channel protein or portion thereof; (2) regulate the phosphorylation state of a potassium channel protein or portion thereof; (3) associate with (*e.g.*, bind) calcium and, for example, act as a calcium dependent kinase, *e.g.*, phosphorylate a potassium channel in a calcium-dependent manner; (4) associate  
25 with (*e.g.*, bind) calcium and, for example, act as a calcium dependent transcription factor; (5) modulate a potassium channel mediated activity in a cell (*e.g.*, a neuronal or cardiac cell) to, for example, beneficially affect the cell; (6) modulate the release of neurotransmitters; (7) modulate membrane excitability; (8) influence the resting potential of membranes; (9) modulate wave forms and frequencies of action potentials;  
30 and (10) modulate thresholds of excitation.

In addition to the nucleic acid molecules encoding PCIP proteins described above, another aspect of the invention pertains to isolated nucleic acid molecules which are antisense thereto. An "antisense" nucleic acid comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, *e.g.*,

5 complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire PCIP coding strand, or to only a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the  
10 coding strand of a nucleotide sequence encoding PCIP. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues. In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding PCIP. The term "noncoding region" refers to 5' and 3' sequences which flank  
15 the coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding PCIP disclosed herein, antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the  
20 entire coding region of PCIP mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of PCIP mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of PCIP mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An  
25 antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of  
30 the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-

fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 5 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from 10 the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a PCIP protein to thereby inhibit expression of the 20 protein, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention include direct 25 injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or 30 antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the

antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an  $\alpha$ -anomeric nucleic acid molecule. An  $\alpha$ -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual  $\beta$ -units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-O-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett.* 215:327-330).

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave PCIP mRNA transcripts to thereby inhibit translation of PCIP mRNA. A ribozyme having specificity for a PCIP-encoding nucleic acid can be designed based upon the nucleotide sequence of a PCIP cDNA disclosed herein (*i.e.*, SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a PCIP-encoding mRNA. See, *e.g.*, Cech *et al.* U.S. Patent



No. 4,987,071; and Cech *et al.* U.S. Patent No. 5,116,742. Alternatively, PCIP mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

5        Alternatively, PCIP gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the PCIP (*e.g.*, the PCIP promoter and/or enhancers) to form triple helical structures that prevent transcription of the PCIP gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6(6):569-84; Helene, C. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992)  
10   *Bioassays* 14(12):807-15.

      In yet another embodiment, the PCIP nucleic acid molecules of the present invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to  
15   generate peptide nucleic acids (see Hyrup B. *et al.* (1996) *Bioorganic & Medicinal Chemistry* 4 (1): 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific  
20   hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *et al.* *Proc. Natl. Acad. Sci.* 93: 14670-675.

      PNAs of PCIP nucleic acid molecules can be used in therapeutic and diagnostic  
25   applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of PCIP nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (*e.g.*, by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination  
30   with other enzymes, (*e.g.*, S1 nucleases (Hyrup B. (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *supra*).

In another embodiment, PNAs of PCIP can be modified, (*e.g.*, to enhance their stability or cellular uptake), by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of PCIP nucleic acid

5 molecules can be generated which may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, (*e.g.*, RNase H and DNA polymerases), to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the  
10 nucleobases, and orientation (Hyrup B. (1996) *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup B. (1996) *supra* and Finn P.J. *et al.* (1996) *Nucleic Acids Res.* 24 (17): 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine  
15 phosphoramidite, can be used as a between the PNA and the 5' end of DNA (Mag, M. *et al.* (1989) *Nucleic Acid Res.* 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn P.J. *et al.* (1996) *supra*). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment (Peterser, K.H. *et al.* (1975)  
20 *Bioorganic Med. Chem. Lett.* 5: 1119-11124).

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.* (1989) *Proc. Natl. Acad. Sci. US.* 86:6553-6556; Lemaitre *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652;  
25 PCT Publication No. W088/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, *e.g.*, Krol *et al.* (1988) *Bio-Techniques* 6:958-976) or intercalating agents. (See, *e.g.*, Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (*e.g.*, a peptide, hybridization  
30 triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

## II. Isolated PCIP Proteins and Anti-PCIP Antibodies

One aspect of the invention pertains to isolated PCIP proteins, and biologically active portions thereof, as well as polypeptide fragments suitable for use as immunogens to raise anti-PCIP antibodies. In one embodiment, native PCIP proteins can be isolated  
5 from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, PCIP proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, a PCIP protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" protein or biologically active portion thereof is  
10 substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the PCIP protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of PCIP protein in which the protein is separated from cellular components of the cells from which it is isolated or  
15 recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of PCIP protein having less than about 30% (by dry weight) of non-PCIP protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-PCIP protein, still more preferably less than about 10% of non-PCIP protein, and most preferably less than about 5% non-PCIP  
20 protein. When the PCIP protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

The language "substantially free of chemical precursors or other chemicals"  
25 includes preparations of PCIP protein in which the protein is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of PCIP protein having less than about 30% (by dry weight) of  
chemical precursors or non-PCIP chemicals, more preferably less than about 20%  
30 chemical precursors or non-PCIP chemicals, still more preferably less than about 10% chemical precursors or non-PCIP chemicals, and most preferably less than about 5% chemical precursors or non-PCIP chemicals.

As used herein, a "biologically active portion" of a PCIP protein includes a fragment of a PCIP protein which participates in an interaction between a PCIP molecule and a non-PCIP molecule. Biologically active portions of a PCIP protein include peptides comprising amino acid sequences sufficiently identical to or derived from the amino acid sequence of the PCIP protein, *e.g.*, the amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109, which include less amino acids than the full length PCIP proteins, and exhibit at least one activity of a PCIP protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the PCIP protein, *e.g.*, binding of a potassium channel subunit. A biologically active portion of a PCIP protein can be a polypeptide which is, for example, 10, 25, 50, 100, 200, or more amino acids in length. Biologically active portions of a PCIP protein can be used as targets for developing agents which modulate a potassium channel mediated activity.

In one embodiment, a biologically active portion of a PCIP protein comprises at least one calcium binding domain.

It is to be understood that a preferred biologically active portion of a PCIP protein of the present invention may contain at least one of the above-identified structural domains. A more preferred biologically active portion of a PCIP protein may contain at least two of the above-identified structural domains. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native PCIP protein.

In a preferred embodiment, the PCIP protein has an amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109. In other embodiments, the PCIP protein is substantially homologous to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109, and retains the functional activity of the protein of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail in subsection I above. Accordingly, in another embodiment, the PCIP protein is a protein which comprises an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more identical to SEQ ID

NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109.

- 10 Isolated proteins of the present invention, preferably 1v, 9q, p19, W28559, KChIP4a, KChIP4b, 33b07, 1p, or 7s proteins, have an amino acid sequence sufficiently identical to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, 15 SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109 or are encoded by a nucleotide sequence sufficiently 20 identical to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, 25 SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102. As used herein, the term "sufficiently 30 identical" refers to a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (*e.g.*, an amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide

sequence such that the first and second amino acid or nucleotide sequences share common structural domains or motifs and/or a common functional activity. For example, amino acid or nucleotide sequences which share common structural domains have at least 30%, 40%, or 50% identity, preferably 60% identity, more preferably 70%-80%, and even more preferably 90-95% identity across the amino acid sequences of the domains and contain at least one and preferably two structural domains or motifs, are defined herein as sufficiently identical. Furthermore, amino acid or nucleotide sequences which share at least 30%, 40%, or 50%, preferably 60%, more preferably 70-80%, or 90-95% identity and share a common functional activity are defined herein as sufficiently identical.

Preferred proteins are PCIP proteins having at least one calcium binding domain and, preferably, a PCIP activity. Other preferred proteins are PCIP proteins having at least one calcium binding domain, and are, preferably, encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102.

To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence (*e.g.*, when aligning a second sequence to the PCIP amino acid sequence of SEQ ID NO:2, SEQ ID NO:4,

SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109 having 177 amino acid residues, at least 80, preferably at least 100, more preferably at least 120, even more preferably at least 140, and even more preferably at least 150, 160 or 170 amino acid residues are aligned). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a Blosum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller (CABIOS, 4:11-17 (1989)) which has been



incorporated into the ALIGN program (version 2.0 or 2.0U), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to PCIP nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to PCIP protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

The invention also provides PCIP chimeric or fusion proteins. As used herein, a PCIP "chimeric protein" or "fusion protein" comprises a PCIP polypeptide operatively linked to a non-PCIP polypeptide. An "PCIP polypeptide" refers to a polypeptide having an amino acid sequence corresponding to PCIP, whereas a "non-PCIP polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the PCIP protein, *e.g.*, a protein which is different from the PCIP protein and which is derived from the same or a different organism. Within a PCIP fusion protein the PCIP polypeptide can correspond to all or a portion of a PCIP protein. In a preferred embodiment, a PCIP fusion protein comprises at least one biologically active portion of a PCIP protein. In another preferred embodiment, a PCIP fusion protein comprises at least two biologically active portions of a PCIP protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the PCIP polypeptide and the non-PCIP polypeptide are fused in-frame to each other. The non-PCIP polypeptide can be fused to the N-terminus or C-terminus of the PCIP polypeptide.

For example, in one embodiment, the fusion protein is a GST-PCIP fusion protein in which the PCIP sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant PCIP.

In another embodiment, the fusion protein is a PCIP protein containing a  
5 heterologous signal sequence at its N-terminus. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of PCIP can be increased through use of a heterologous signal sequence.

The PCIP fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. The PCIP fusion  
10 proteins can be used to affect the bioavailability of a PCIP substrate. Use of PCIP fusion proteins may be useful therapeutically for the treatment of potassium channel associated disorders such as CNS disorders, *e.g.*, neurodegenerative disorders such as Alzheimer's disease, dementias related to Alzheimer's disease (such as Pick's disease), Parkinson's and other Lewy diffuse body diseases, multiple sclerosis, amyotrophic  
15 lateral sclerosis, progressive supranuclear palsy, epilepsy, spinocerebellar ataxia, and Jakob-Creutzfeldt disease; psychiatric disorders, *e.g.*, depression, schizophrenic disorders, Korsakoff's psychosis, mania, anxiety disorders, or phobic disorders; learning or memory disorders, *e.g.*, amnesia or age-related memory loss; and neurological disorders; *e.g.*, migraine. Use of PCIP fusion proteins may also be useful therapeutically  
20 for the treatment of potassium channel associated disorders such as cardiovascular disorders, *e.g.*, arteriosclerosis, ischemia reperfusion injury, restenosis, arterial inflammation, vascular wall remodeling, ventricular remodeling, rapid ventricular pacing, coronary microembolism, tachycardia, bradycardia, pressure overload, aortic bending, coronary artery ligation, vascular heart disease, atrial fibrillation or congestive  
25 heart failure.

Moreover, the PCIP-fusion proteins of the invention can be used as immunogens to produce anti-PCIP antibodies in a subject, to purify PCIP ligands and in screening assays to identify molecules which inhibit the interaction of PCIP with a PCIP substrate.

Preferably, a PCIP chimeric or fusion protein of the invention is produced by  
30 standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended

termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers.

- 5 Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel *et al.* John Wiley & Sons: 1992). Moreover, many expression vectors are commercially
- 10 available that already encode a fusion moiety (*e.g.*, a GST polypeptide). A PCIP-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the PCIP protein.

- The present invention also pertains to variants of the PCIP proteins which function as either PCIP agonists (mimetics) or as PCIP antagonists. Variants of the
- 15 PCIP proteins can be generated by mutagenesis, *e.g.*, discrete point mutation or truncation of a PCIP protein. An agonist of the PCIP proteins can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of a PCIP protein. An antagonist of a PCIP protein can inhibit one or more of the activities of the naturally occurring form of the PCIP protein by, for example, competitively
- 20 modulating a potassium channel mediated activity of a PCIP protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the PCIP protein.

- 25 In one embodiment, variants of a PCIP protein which function as either PCIP agonists (mimetics) or as PCIP antagonists can be identified by screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of a PCIP protein for PCIP protein agonist or antagonist activity. In one embodiment, a variegated library of PCIP variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a
- 30 variegated gene library. A variegated library of PCIP variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential PCIP sequences is expressible as

individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of PCIP sequences therein. There are a variety of methods which can be used to produce libraries of potential PCIP variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential PCIP sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, *e.g.*, Narang, S.A. (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu. Rev. Biochem.* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucleic Acid Res.* 11:477.

In addition, libraries of fragments of a PCIP protein coding sequence can be used to generate a variegated population of PCIP fragments for screening and subsequent selection of variants of a PCIP protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of a PCIP coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal, C-terminal and internal fragments of various sizes of the PCIP protein.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of PCIP proteins. The most widely used techniques, which are amenable to high throughput analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique which

enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify PCIP variants (Arkin and Yourvan (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave *et al.* (1993) *Protein Engineering* 6(3):327-331).

- 5           In one embodiment, cell based assays can be exploited to analyze a variegated PCIP library. For example, a library of expression vectors can be transfected into a cell line which ordinarily possesses a potassium channel mediated activity. The effect of the PCIP mutant on the potassium channel mediated activity can then be detected, *e.g.*, by any of a number of enzymatic assays or by detecting the release of a neurotransmitter.
- 10       Plasmid DNA can then be recovered from the cells which score for inhibition, or alternatively, potentiation of the potassium channel mediated activity, and the individual clones further characterized.

- An isolated PCIP protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind PCIP using standard techniques for
- 15       polyclonal and monoclonal antibody preparation. A full-length PCIP protein can be used or, alternatively, the invention provides antigenic peptide fragments of PCIP for use as immunogens. The antigenic peptide of PCIP comprises at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16,
  - 20       SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID
  - 25       NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109 and encompasses an epitope of PCIP such that an antibody raised against the peptide forms a specific immune complex with PCIP. Preferably, the antigenic peptide comprises at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more
  - 30       preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

Preferred epitopes encompassed by the antigenic peptide are regions of PCIP that are located on the surface of the protein, *e.g.*, hydrophilic regions, as well as regions with high antigenicity.

A PCIP immunogen typically is used to prepare antibodies by immunizing a suitable subject, (*e.g.*, rabbit, goat, mouse or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed PCIP protein or a chemically synthesized PCIP polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic PCIP preparation induces a polyclonal anti-PCIP antibody response.

Accordingly, another aspect of the invention pertains to anti-PCIP antibodies. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as PCIP. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab')<sub>2</sub> fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind PCIP. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of PCIP. A monoclonal antibody composition thus typically displays a single binding affinity for a particular PCIP protein with which it immunoreacts.

Polyclonal anti-PCIP antibodies can be prepared as described above by immunizing a suitable subject with a PCIP immunogen. The anti-PCIP antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized PCIP. If desired, the antibody molecules directed against PCIP can be isolated from the mammal (*e.g.*, from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, *e.g.*, when the anti-PCIP antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard

techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497) (see also, Brown *et al.* (1981) *J. Immunol.* 127:539-46; Brown *et al.* (1980) *J. Biol. Chem.* 255:4980-83; Yeh *et al.* (1976) *Proc. Natl. Acad. Sci. USA* 76:2927-31; and Yeh *et al.* (1982) *Int. J. Cancer* 29:269-75), the more recent human B cell hybridoma technique (Kozbor *et al.* (1983) *Immunol Today* 4:72), the EBV-hybridoma technique (Cole *et al.* (1985), *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal antibody hybridomas is well known (see generally R. H. Kenneth, in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); E. A. Lerner (1981) *Yale J. Biol. Med.*, 54:387-402; M. L. Gefter *et al.* (1977) *Somatic Cell Genet.* 3:231-36). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with a PCIP immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds PCIP.

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti-PCIP monoclonal antibody (see, *e.g.*, G. Galfre *et al.* (1977) *Nature* 266:55052; Gefter *et al.* *Somatic Cell Genet.*, cited *supra*; Lerner, *Yale J. Biol. Med.*, cited *supra*; Kenneth, *Monoclonal Antibodies*, cited *supra*). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (*e.g.*, a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can be used as a fusion partner according to standard techniques, *e.g.*, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused

and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind PCIP, *e.g.*, using a standard ELISA assay.

- 5           Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-PCIP antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (*e.g.*, an antibody phage display library) with PCIP to thereby isolate immunoglobulin library members that bind PCIP. Kits for generating and screening phage display libraries are commercially available
- 10   (*e.g.*, the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP™ Phage Display Kit*, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, Ladner *et al.* U.S. Patent No. 5,223,409; Kang *et al.* PCT International Publication No. WO 92/18619;
- 15   Dower *et al.* PCT International Publication No. WO 91/17271; Winter *et al.* PCT International Publication WO 92/20791; Markland *et al.* PCT International Publication No. WO 92/15679; Breitling *et al.* PCT International Publication WO 93/01288; McCafferty *et al.* PCT International Publication No. WO 92/01047; Garrard *et al.* PCT International Publication No. WO 92/09690; Ladner *et al.* PCT International Publication
- 20   No. WO 90/02809; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J. Mol. Biol.* 226:889-896; Clarkson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:3576-3580; Garrard *et al.* (1991) *Bio/Technology* 9:1373-1377;
- 25   Hoogenboom *et al.* (1991) *Nuc. Acid Res.* 19:4133-4137; Barbas *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:7978-7982; and McCafferty *et al.* *Nature* (1990) 348:552-554.

- Additionally, recombinant anti-PCIP antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the
- 30   invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson *et al.* International Application No. PCT/US86/02269; Akira, *et al.* European



- Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison *et al.* European Patent Application 173,494; Neuberger *et al.* PCT International Publication No. WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567; Cabilly *et al.* European Patent Application 125,023; Better *et al.* (1988) *Science* 240:1041-1043; Liu *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu *et al.* (1987) *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:214-218; Nishimura *et al.* (1987) *Canc. Res.* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; and Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80:1553-1559; Morrison, S. L. (1985) *Science* 229:1202-1207; Oi *et al.* (1986) *BioTechniques* 4:214; Winter U.S. Patent 5,225,539; Jones *et al.* (1986) *Nature* 321:552-525; Verhoeyan *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J. Immunol.* 141:4053-4060.

An anti-PCIP antibody (*e.g.*, monoclonal antibody) can be used to isolate PCIP by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-PCIP antibody can facilitate the purification of natural PCIP from cells and of recombinantly produced PCIP expressed in host cells. Moreover, an anti-PCIP antibody can be used to detect PCIP protein (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the PCIP protein. Anti-PCIP antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{35}\text{S}$  or  $^3\text{H}$ .

### III. Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding a PCIP protein (or a portion thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting  
5 another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial  
10 vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression  
15 vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-  
20 associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is  
25 operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory  
30 sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185,

Academic Press, San Diego, CA (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cell and those which direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, PCIP proteins, mutant forms of PCIP proteins, fusion proteins, and the like).

The recombinant expression vectors of the invention can be designed for expression of PCIP proteins in prokaryotic or eukaryotic cells. For example, PCIP proteins can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA)

and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Purified fusion proteins can be utilized in PCIP activity assays, (*e.g.*, direct assays or competitive assays described in detail below), or to generate antibodies specific for PCIP proteins, for example. In a preferred embodiment, a PCIP fusion protein expressed in a retroviral expression vector of the present invention can be utilized to infect bone marrow cells which are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (*e.g.*, six (6) weeks).

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 60-89). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter.

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada *et al.*, (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the PCIP expression vector is a yeast expression vector.

Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari, *et al.*, (1987) *Embo J.* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-

943), pJRY88 (Schultz *et al.*, (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, CA), and picZ (Invitrogen Corp, San Diego, CA).

Alternatively, PCIP proteins can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured  
5 insect cells (*e.g.*, Sf 9 cells) include the pAc series (Smith *et al.* (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B. (1987) *Nature* 329:840) and pMT2PC  
10 (Kaufman *et al.* (1987) *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J., Fritsh, E. F.,  
15 and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type  
20 (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.* (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and  
25 Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle (1989) *Proc. Natl. Acad. Sci. USA* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Patent No.  
30 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example the murine hox promoters

(Kessel and Gruss (1990) *Science* 249:374-379) and the  $\alpha$ -fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to PCIP mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. *et al.*, Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, a PCIP protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized

techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (*Molecular Cloning: A*  
5 *Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989*), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these  
10 integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding a PCIP protein or can be  
15 introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) a PCIP protein. Accordingly, the  
20 invention further provides methods for producing a PCIP protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding a PCIP protein has been introduced) in a suitable medium such that a PCIP protein is produced. In another embodiment, the method further comprises isolating a PCIP protein from the medium or  
25 the host cell.

The host cells of the invention can also be used to produce non-human transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which PCIP-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in  
30 which exogenous PCIP sequences have been introduced into their genome or homologous recombinant animals in which endogenous PCIP sequences have been altered. Such animals are useful for studying the function and/or activity of a PCIP and

for identifying and/or evaluating modulators of PCIP activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, and the like. A transgene is exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous PCIP gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing a PCIP-encoding nucleic acid into the male pronuclei of a fertilized oocyte, *e.g.*, by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The PCIP cDNA sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102 can be introduced as a transgene into the genome of a non-human animal. Alternatively, a nonhuman homologue of a human PCIP gene, such as a mouse or rat PCIP gene, can be used as a transgene. Alternatively, a PCIP gene homologue, such as another PCIP family member, can be isolated based on hybridization to the PCIP cDNA sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ



ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102 or the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994 (described further in subsection I above) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to a PCIP transgene to direct expression of a PCIP protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, both by Leder *et al.*, U.S. Patent No. 4,873,191 by Wagner *et al.* and in Hogan, B., *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of a PCIP transgene in its genome and/or expression of PCIP mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding a PCIP protein can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of a PCIP gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the PCIP gene. The PCIP gene can be a human gene (*e.g.*, the cDNA of SEQ ID NO:1), but more preferably, is a non-human homologue of a human PCIP gene (*e.g.*, the cDNA of SEQ ID NO:3 or 5). For example, a mouse PCIP gene can be used to construct a homologous recombination vector suitable for altering an endogenous PCIP gene in the mouse genome. In a

preferred embodiment, the vector is designed such that, upon homologous recombination, the endogenous PCIP gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector). Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous

5 PCIP gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous PCIP protein). In the homologous recombination vector, the altered portion of the PCIP gene is flanked at its 5' and 3' ends by additional nucleic acid sequence of the PCIP gene to allow for homologous recombination to occur between the exogenous

10 PCIP gene carried by the vector and an endogenous PCIP gene in an embryonic stem cell. The additional flanking PCIP nucleic acid sequence is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the vector (see *e.g.*, Thomas, K.R. and Capecchi, M. R. (1987) *Cell* 51:503 for a description of

15 homologous recombination vectors). The vector is introduced into an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced PCIP gene has homologously recombined with the endogenous PCIP gene are selected (see *e.g.*, Li, E. *et al.* (1992) *Cell* 69:915). The selected cells are then injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras (see *e.g.*, Bradley, A. in

20 *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E.J. Robertson, ed. (IRL, Oxford, 1987) pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by

25 germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley, A. (1991) *Current Opinion in Biotechnology* 2:823-829 and in PCT International Publication Nos.: WO 90/11354 by Le Mouellec *et al.*; WO 91/01140 by Smithies *et al.*; WO 92/0968 by Zijlstra *et al.*; and WO 93/04169 by Berns *et al.*

30 In another embodiment, transgenic non-humans animals can be produced which contain selected systems which allow for regulated expression of the transgene. One example of such a system is the *cre/loxP* recombinase system of bacteriophage P1. For

a description of the *cre/loxP* recombinase system, see, *e.g.*, Lakso *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman *et al.* (1991) *Science* 251:1351-1355. If a *cre/loxP* recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the *Cre* recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, *e.g.*, by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, I. *et al.* (1997) *Nature* 385:810-813 and PCT International Publication Nos. WO 97/07668 and WO 97/07669. In brief, a cell, *e.g.*, a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G<sub>0</sub> phase. The quiescent cell can then be fused, *e.g.*, through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, *e.g.*, the somatic cell, is isolated.

#### IV. Pharmaceutical Compositions

The PCIP nucleic acid molecules, fragments of PCIP proteins, and anti-PCIP antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof

in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include  
5 parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents;  
10 antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be  
15 enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For  
20 intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as  
25 bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of  
30 surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include

isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

5 Sterile injectable solutions can be prepared by incorporating the active compound (*e.g.*, a fragment of a PCIP protein or an anti-PCIP antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic  
10 dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

15 Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is  
20 applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or  
25 lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an  
30 aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

As defined herein, a therapeutically effective amount of protein or polypeptide (*i.e.*, an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide, or antibody can include a single treatment or, preferably, can include a series of treatments.

In a preferred example, a subject is treated with antibody, protein, or polypeptide in the range of between about 0.1 to 20 mg/kg body weight, one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of antibody, protein, or polypeptide used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result and become apparent from the results of diagnostic assays as described herein.

The present invention encompasses agents which modulate expression or activity. An agent may, for example, be a small molecule. For example, such small molecules include, but are not limited to, peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (i.e., including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

It is understood that appropriate doses of small molecule agents depends upon a number of factors within the ken of the ordinarily skilled physician, veterinarian, or researcher. The dose(s) of the small molecule will vary, for example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner desires the small molecule to have upon the nucleic acid or polypeptide of the invention.

Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (e.g., about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram. It is furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated.



Such appropriate doses may be determined using the assays described herein. When one or more of these small molecules is to be administered to an animal (*e.g.*, a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

Further, an antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as

tumor necrosis factor, alpha.-interferon, beta.-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"),  
5 granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, *e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug  
10 Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in  
15 *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.*, 62:119-58 (1982). Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S.  
20 Patent No. 4,676,980.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see *e.g.*, Chen *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-  
25 3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene  
30 delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

## V. Uses and Methods of the Invention

The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: a) screening assays; b) predictive medicine (*e.g.*, diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenetics); and c) methods of treatment (*e.g.*, therapeutic and prophylactic). As described herein, a PCIP protein of the invention has one or more of the following activities: (1) it interacts with (*e.g.*, binds to) a potassium channel protein or portion thereof; (2) it regulates the phosphorylation state of a potassium channel protein or portion thereof; (3) it associates with (*e.g.*, binds to) calcium and can, for example, act as a calcium dependent kinase, *e.g.*, phosphorylate a potassium channel or a G-protein coupled receptor in a calcium-dependent manner; (4) it associates with (*e.g.*, binds to) calcium and can, for example, act as a calcium dependent transcription factor; (5) it modulates a potassium channel mediated activity in a cell (*e.g.*, a neuronal or cardiac cell) to, for example, beneficially affect the cell; (6) it modulates chromatin formation in a cell, *e.g.*, a neuronal or cardiac cell; (7) it modulates vesicular traffic and protein transport in a cell, *e.g.*, a neuronal or cardiac cell; (8) it modulates cytokine signaling in a cell, *e.g.*, a neuronal or cardiac cell; (9) it regulates the association of a potassium channel protein or portion thereof with the cellular cytoskeleton; (10) it modulates cellular proliferation; (11) it modulates the release of neurotransmitters; (12) it modulates membrane excitability; (13) it influences the resting potential of membranes; (14) it modulates wave forms and frequencies of action potentials; and (15) it modulates thresholds of excitation and, thus, can be used to, for example, (1) modulate the activity of a potassium channel protein or portion thereof; (2) modulate the phosphorylation state of a potassium channel protein or portion thereof; (3) modulate the phosphorylation state of a potassium channel or a G-protein coupled receptor in a calcium-dependent manner; (4) associate with (*e.g.*, bind to) calcium and act as a calcium dependent transcription factor; (5) modulate a potassium channel mediated activity in a cell (*e.g.*, a neuronal or cardiac cell) to, for example, beneficially affect the cell; (6) modulate chromatin formation in a cell, *e.g.*, a neuronal or cardiac cell; (7) modulate vesicular traffic and protein transport in a cell, *e.g.*, a neuronal or cardiac cell; (8) modulate cytokine signaling in a cell, *e.g.*, a neuronal or cardiac cell; (9) regulate the

- association of a potassium channel protein or portion thereof with the cellular cytoskeleton; (10) modulate cellular proliferation; (11) modulate the release of neurotransmitters; (12) modulate membrane excitability; (13) influence the resting potential of membranes; (14) modulate wave forms and frequencies of action potentials; and (15) modulate thresholds of excitation.

The isolated nucleic acid molecules of the invention can be used, for example, to express PCIP protein (*e.g.*, via a recombinant expression vector in a host cell in gene therapy applications), to detect PCIP mRNA (*e.g.*, in a biological sample) or a genetic alteration in a PCIP gene, and to modulate PCIP activity, as described further below.

- 10 The PCIP proteins can be used to treat disorders characterized by insufficient or excessive production of a PCIP substrate or production of PCIP inhibitors. In addition, the PCIP proteins can be used to screen for naturally occurring PCIP substrates, to screen for drugs or compounds which modulate PCIP activity, as well as to treat disorders characterized by insufficient or excessive production of PCIP protein or
- 15 production of PCIP protein forms which have decreased or aberrant activity compared to PCIP wild type protein (*e.g.*, CNS disorders such as neurodegenerative disorders, *e.g.*, Alzheimer's disease, dementias related to Alzheimer's disease (such as Pick's disease), Parkinson's and other Lewy diffuse body diseases, multiple sclerosis, amyotrophic lateral sclerosis, progressive supranuclear palsy, epilepsy, spinocerebellar ataxia, and
- 20 Jakob-Creutzfeldt disease; psychiatric disorders, *e.g.*, depression, schizophrenic disorders, Korsakoff's psychosis, mania, anxiety disorders, bipolar affective disorders, or phobic disorders; learning or memory disorders, *e.g.*, amnesia or age-related memory loss; neurological disorders, *e.g.*, migraine; pain disorders, *e.g.*, hyperalgesia or pain associated with musculoskeletal disorders; spinal cord injury; stroke; and head trauma;
- 25 or cardiovascular disorders such as sinus node dysfunction, angina, heart failure, hypertension, atrial fibrillation, atrial flutter, dilated cardiomyopathy, idiopathic cardiomyopathy, myocardial infarction, coronary artery disease, coronary artery spasm, or arrhythmia). Moreover, the anti-PCIP antibodies of the invention can be used to detect and isolate PCIP proteins, regulate the bioavailability of PCIP proteins, and modulate
- 30 PCIP activity.

A. Screening Assays:

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) which bind to PCIP proteins, have a stimulatory or inhibitory effect on, for example, PCIP expression or PCIP activity, or have a stimulatory or inhibitory effect on, for example, the expression or activity of PCIP substrate.

In one embodiment, the invention provides assays for screening candidate or test compounds which are substrates of a PCIP protein or polypeptide or biologically active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of a PCIP protein or polypeptide or biologically active portion thereof. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994) *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP '409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406);

(Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici (1991) *J. Mol. Biol.* 222:301-310); (Ladner *supra.*).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a PCIP protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate PCIP activity, *e.g.*, binding to a potassium channel or a portion thereof, is determined. Determining the ability of the test compound to modulate PCIP activity can be accomplished by monitoring, for example, the release of a neurotransmitter, *e.g.*, dopamine, from a cell which expresses PCIP such as a neuronal cell, *e.g.*, a substantia nigra neuronal cell, or a cardiac cell.

Furthermore, determining the ability of the test compound to modulate PCIP activity can be accomplished by monitoring, for example, the  $I_o$  current or the release of a neurotransmitter from a cell which expresses PCIP such as a cardiac cell. Currents in cells, *e.g.*, the  $I_o$  current, can be measured using the patch-clamp technique as described in the Examples section using the techniques described in, for example, Hamill *et al.* 1981. *Pfluegers Arch.* 391: 85-100). The cell, for example, can be of mammalian origin. Determining the ability of the test compound to modulate the ability of PCIP to bind to a substrate can be accomplished, for example, by coupling the PCIP substrate with a radioisotope or enzymatic label such that binding of the PCIP substrate to PCIP can be determined by detecting the labeled PCIP substrate in a complex. For example, compounds (*e.g.*, PCIP substrates) can be labeled with  $^{125}\text{I}$ ,  $^{35}\text{S}$ ,  $^{14}\text{C}$ , or  $^3\text{H}$ , either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

It is also within the scope of this invention to determine the ability of a compound (*e.g.*, PCIP substrate) to interact with PCIP without the labeling of any of the interactants. For example, a microphysiometer can be used to detect the interaction of a compound with PCIP without the labeling of either the compound or the PCIP.

McConnell, H. M. *et al.* (1992) *Science* 257:1906-1912. As used herein, a "microphysiometer" (*e.g.*, Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor

(LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a compound and PCIP.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a PCIP target molecule (*e.g.*, a potassium channel or a fragment thereof) with a test compound and determining the ability of the test compound to modulate (*e.g.* stimulate or inhibit) the activity of the PCIP target molecule. Determining the ability of the test compound to modulate the activity of a PCIP target molecule can be accomplished, for example, by determining the ability of the PCIP protein to bind to or interact with the PCIP target molecule, *e.g.*, a potassium channel or a fragment thereof.

Determining the ability of the PCIP protein or a biologically active fragment thereof, to bind to or interact with a PCIP target molecule can be accomplished by one of the methods described above for determining direct binding. In a preferred embodiment, determining the ability of the PCIP protein to bind to or interact with a PCIP target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.*, intracellular  $\text{Ca}^{2+}$ , diacylglycerol,  $\text{IP}_3$ , and the like), detecting catalytic/enzymatic activity of the target an appropriate substrate, detecting the induction of a reporter gene (comprising a target-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a target-regulated cellular response such as the release of a neurotransmitter.

In yet another embodiment, an assay of the present invention is a cell-free assay in which a PCIP protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the PCIP protein or biologically active portion thereof is determined. Preferred biologically active portions of the PCIP proteins to be used in assays of the present invention include fragments which participate in interactions with non-PCIP molecules, *e.g.*, potassium channels or fragments thereof, or fragments with high surface probability scores. Binding of the test compound to the PCIP protein can be determined either directly or indirectly as described above. In a preferred embodiment, the assay includes contacting the PCIP protein or biologically active portion thereof with a known compound which binds PCIP to form an assay mixture, contacting the assay mixture with a test compound, and

determining the ability of the test compound to interact with a PCIP protein, wherein determining the ability of the test compound to interact with a PCIP protein comprises determining the ability of the test compound to preferentially bind to PCIP or biologically active portion thereof as compared to the known compound.

5           In another embodiment, the assay is a cell-free assay in which a PCIP protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the PCIP protein or biologically active portion thereof is determined. Determining the ability of the test compound to modulate the activity of a PCIP protein can be accomplished, for example,  
10 by determining the ability of the PCIP protein to bind to a PCIP target molecule by one of the methods described above for determining direct binding. Determining the ability of the PCIP protein to bind to a PCIP target molecule can also be accomplished using a technology such as real-time Biomolecular Interaction Analysis (BIA). Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo *et al.* (1995) *Curr.*  
15 *Opin. Struct. Biol.* 5:699-705. As used herein, "BIA" is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore). Changes in the optical phenomenon of surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

          In an alternative embodiment, determining the ability of the test compound to  
20 modulate the activity of a PCIP protein can be accomplished by determining the ability of the PCIP protein to further modulate the activity of a downstream effector of a PCIP target molecule. For example, the activity of the effector molecule on an appropriate target can be determined or the binding of the effector to an appropriate target can be determined as previously described.

25           In yet another embodiment, the cell-free assay involves contacting a PCIP protein or biologically active portion thereof with a known compound which binds the PCIP protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the PCIP protein, wherein determining the ability of the test compound to interact with the PCIP  
30 protein comprises determining the ability of the PCIP protein to preferentially bind to or modulate the activity of a PCIP target molecule.



The cell-free assays of the present invention are amenable to use of both soluble and/or membrane-bound forms of isolated proteins. In the case of cell-free assays in which a membrane-bound form of an isolated protein is used (*e.g.*, a potassium channel) it may be desirable to utilize a solubilizing agent such that the membrane-bound form of the isolated protein is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton<sup>®</sup> X-100, Triton<sup>®</sup> X-114, Thesit<sup>®</sup>, Isotridecypoly(ethylene glycol ether)<sub>n</sub>, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-[(3-cholamidopropyl)dimethylamminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl=N,N-dimethyl-3-ammonio-1-propane sulfonate.

In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either PCIP or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to a PCIP protein, or interaction of a PCIP protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/ PCIP fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtitre plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or PCIP protein, and the mixture incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtitre plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of PCIP binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either a PCIP protein or a PCIP target molecule can be immobilized utilizing conjugation of biotin and streptavidin.

5 Biotinylated PCIP protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with PCIP protein or target molecules but which do not interfere with binding of the PCIP protein to its target molecule can be derivatized to the wells of the plate, and unbound target or PCIP protein  
10 trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the PCIP protein or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the PCIP protein or target molecule.

15 In a preferred embodiment, candidate or test compounds or agents are tested for their ability to inhibit or stimulate a PCIP molecule's ability to modulate vesicular traffic and protein transport in a cell, *e.g.*, a neuronal or cardiac cell, using the assays described in, for example, Komada M. *et al.* (1999) *Genes Dev.* 13(11):1475-85, and Roth M.G. *et al.* (1999) *Chem. Phys. Lipids.* 98(1-2):141-52, the contents of which are incorporated  
20 herein by reference.

In another preferred embodiment, candidate or test compounds or agents are tested for their ability to inhibit or stimulate a PCIP molecule's ability to regulate the phosphorylation state of a potassium channel protein or portion thereof, using for example, an *in vitro* kinase assay. Briefly, a PCIP target molecule, *e.g.*, an  
25 immunoprecipitated potassium channel from a cell line expressing such a molecule, can be incubated with the PCIP protein and radioactive ATP, *e.g.*, [ $\gamma$ -<sup>32</sup>P] ATP, in a buffer containing MgCl<sub>2</sub> and MnCl<sub>2</sub>, *e.g.*, 10 mM MgCl<sub>2</sub> and 5 mM MnCl<sub>2</sub>. Following the incubation, the immunoprecipitated PCIP target molecule, *e.g.*, the potassium channel, can be separated by SDS-polyacrylamide gel electrophoresis under reducing conditions,  
30 transferred to a membrane, *e.g.*, a PVDF membrane, and autoradiographed. The appearance of detectable bands on the autoradiograph indicates that the PCIP substrate, *e.g.*, the potassium channel, has been phosphorylated. Phosphoaminoacid analysis of the

phosphorylated substrate can also be performed in order to determine which residues on the PCIP substrate are phosphorylated. Briefly, the radiophosphorylated protein band can be excised from the SDS gel and subjected to partial acid hydrolysis. The products can then be separated by one-dimensional electrophoresis and analyzed on, for example, a phosphoimager and compared to ninhydrin-stained phosphoaminoacid standards. Assays such as those described in, for example, Tamaskovic R. *et al.* (1999) *Biol. Chem.* 380(5):569-78, the contents of which are incorporated herein by reference, can also be used.

In another preferred embodiment, candidate or test compounds or agents are tested for their ability to inhibit or stimulate a PCIP molecule's ability to associate with (e.g., bind) calcium, using for example, the assays described in Liu L. (1999) *Cell Signal.* 11(5):317-24 and Kawai T. *et al.* (1999) *Oncogene* 18(23):3471-80, the contents of which are incorporated herein by reference.

In another preferred embodiment, candidate or test compounds or agents are tested for their ability to inhibit or stimulate a PCIP molecule's ability to modulate chromatin formation in a cell, using for example, the assays described in Okuwaki M. *et al.* (1998) *J. Biol. Chem.* 273(51):34511-8 and Miyaji-Yamaguchi M. (1999) *J. Mol. Biol.* 290(2): 547-557, the contents of which are incorporated herein by reference.

In yet another preferred embodiment, candidate or test compounds or agents are tested for their ability to inhibit or stimulate a PCIP molecule's ability to modulate cellular proliferation, using for example, the assays described in Baker F.L. *et al.* (1995) *Cell Prolif.* 28(1):1-15, Cheviron N. *et al.* (1996) *Cell Prolif.* 29(8):437-46, Hu Z.W. *et al.* (1999) *J. Pharmacol. Exp. Ther.* 290(1):28-37 and Elliott K. *et al.* (1999) *Oncogene* 18(24):3564-73, the contents of which are incorporated herein by reference.

In a preferred embodiment, candidate or test compounds or agents are tested for their ability to inhibit or stimulate a PCIP molecule's ability to regulate the association of a potassium channel protein or portion thereof with the cellular cytoskeleton, using for example, the assays described in Gonzalez C. *et al.* (1998) *Cell Mol. Biol.* 44(7):1117-27 and Chia C.P. *et al.* (1998) *Exp. Cell Res.* 244(1):340-8, the contents of which are incorporated herein by reference.

In another preferred embodiment, candidate or test compounds or agents are tested for their ability to inhibit or stimulate a PCIP molecule's ability to modulate membrane excitability, using for example, the assays described in Bar-Sagi D. *et al.* (1985) *J. Biol. Chem.* 260(8):4740-4 and Barker J.L. *et al.* (1984) *Neurosci. Lett.*

5 47(3):313-8, the contents of which are incorporated herein by reference.

In another preferred embodiment, candidate or test compounds or agents are tested for their ability to inhibit or stimulate a PCIP molecule's ability to modulate cytokine signaling in a cell, *e.g.*, a neuronal or cardiac cell, the assays described in Nakashima Y. *et al.* (1999) *J. Bone Joint Surg. Am.* 81(5):603-15, the contents of which

10 are incorporated herein by reference.

In another embodiment, modulators of PCIP expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of PCIP mRNA or protein in the cell is determined. The level of expression of PCIP mRNA or protein in the presence of the candidate compound is compared to the level of expression of PCIP mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of PCIP expression based on this comparison. For example, when expression of PCIP mRNA or protein is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of PCIP mRNA or protein

15 expression. Alternatively, when expression of PCIP mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of PCIP mRNA or protein expression. The level of PCIP mRNA or protein expression in the cells can be determined by methods described herein for detecting PCIP mRNA or protein.

25 In yet another aspect of the invention, the PCIP proteins can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent No. 5,283,317; Zervos *et al.* (1993) *Cell* 72:223-232; Madura *et al.* (1993) *J. Biol. Chem.* 268:12046-12054; Bartel *et al.* (1993) *Biotechniques* 14:920-924; Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other proteins, which bind to or interact with PCIP ("PCIP-binding proteins" or "PCIP-bp") and are involved in PCIP activity (described in more detail in the Examples section below). Such PCIP-binding proteins are also likely to be involved in the propagation of signals

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by the PCIP proteins or PCIP targets as, for example, downstream elements of a PCIP-mediated signaling pathway. Alternatively, such PCIP-binding proteins are likely to be PCIP inhibitors.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a PCIP protein is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming a PCIP-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the protein which interacts with the PCIP protein.

This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein in an appropriate animal model. For example, an agent identified as described herein (*e.g.*, a PCIP modulating agent, an antisense PCIP nucleic acid molecule, a PCIP-specific antibody, or a PCIP-binding partner) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments, *e.g.*, treatments of a CNS disorder or a cardiovascular disorder, as described herein.

## B. Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) map their  
5 respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.

### 10 1. Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the PCIP  
nucleotide sequences, described herein, can be used to map the location of the PCIP  
15 genes on a chromosome. The mapping of the PCIP sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Briefly, PCIP genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the PCIP nucleotide sequences. Computer analysis of the PCIP sequences can be used to predict primers that do not span more than one  
20 exon in the genomic DNA, thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the PCIP sequences will yield an amplified fragment.

Somatic cell hybrids are prepared by fusing somatic cells from different  
25 mammals (*e.g.*, human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but human cells can, the one human chromosome that contains the gene encoding the needed enzyme, will be retained. By using various media, panels  
30 of hybrid cell lines can be established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human

chromosomes. (D'Eustachio.P. *et al.* (1983) *Science* 220:919-924). Somatic cell hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the PCIP nucleotide sequences to design oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes. Other mapping strategies which can similarly be used to map a PCIP sequence to its chromosome include *in situ* hybridization (described in Fan, Y. *et al.* (1990) *Proc. Natl. Acad. Sci. USA*, 87:6223-27), pre-screening with labeled flow-sorted chromosomes, and pre-selection by hybridization to chromosome specific cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical such as colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases will suffice to get good results at a reasonable amount of time. For a review of this technique, see Verma *et al.*, Human Chromosomes: A Manual of Basic Techniques (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man, available on-line through Johns Hopkins University Welch Medical Library). The relationship between a gene and a disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, for example, Egeland, J. *et al.* (1987) *Nature*, 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the PCIP gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

## 2. Tissue Typing

The PCIP sequences of the present invention can also be used to identify individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The sequences of the present invention are useful as additional DNA markers for RFLP (described in U.S. Patent 5,272,057).

Furthermore, the sequences of the present invention can be used to provide an alternative technique which determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the PCIP nucleotide sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the



sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the present invention can be used to obtain such identification sequences from individuals and from tissue. The PCIP nucleotide sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. Non-coding sequences can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers which each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

If a panel of reagents from PCIP nucleotide sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database, positive identification of the individual, living or dead, can be made from extremely small tissue samples.

### 3. Use of Partial PCIP Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology. Forensic biology is a scientific field employing genetic typing of biological evidence found at a crime scene as a means for positively identifying, for example, a perpetrator of a crime. To make such an identification, PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, *e.g.*, hair or skin, or body fluids, *e.g.*, blood, saliva, or semen found at a crime scene. The amplified

sequence can then be compared to a standard, thereby allowing identification of the origin of the biological sample.

The sequences of the present invention can be used to provide polynucleotide reagents, *e.g.*, PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (*i.e.* another DNA sequence that is unique to a particular individual). As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Sequences targeted to noncoding regions are particularly appropriate for this use as greater numbers of polymorphisms occur in the noncoding regions, making it easier to differentiate individuals using this technique. Examples of polynucleotide reagents include the PCIP nucleotide sequences or portions thereof, having a length of at least 20 bases, preferably at least 30 bases.

The PCIP nucleotide sequences described herein can further be used to provide polynucleotide reagents, *e.g.*, labeled or labelable probes which can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue, *e.g.*, brain tissue. This can be very useful in cases where a forensic pathologist is presented with a tissue of unknown origin. Panels of such PCIP probes can be used to identify tissue by species and/or by organ type.

In a similar fashion, these reagents, *e.g.*, PCIP primers or probes can be used to screen tissue culture for contamination (*i.e.* screen for the presence of a mixture of different types of cells in a culture).

#### C. Predictive Medicine:

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining PCIP protein and/or nucleic acid expression as well as PCIP activity, in the context of a biological sample (*e.g.*, blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant PCIP expression or activity. The invention also

provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with PCIP protein, nucleic acid expression or activity. For example, mutations in a PCIP gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically

5 treat an individual prior to the onset of a disorder characterized by or associated with PCIP protein, nucleic acid expression or activity.

Another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of PCIP in clinical trials.

These and other agents are described in further detail in the following sections.

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#### 1. Diagnostic Assays

An exemplary method for detecting the presence or absence of PCIP protein or nucleic acid in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of

15 detecting PCIP protein or nucleic acid (*e.g.*, mRNA, genomic DNA) that encodes PCIP protein such that the presence of PCIP protein or nucleic acid is detected in the biological sample. A preferred agent for detecting PCIP mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to PCIP mRNA or genomic DNA. The nucleic acid probe can be, for example, a full-length PCIP nucleic acid, such as the

20 nucleic acid of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID

25 NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102, or the DNA insert of the plasmid deposited with ATCC as

30 Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500

nucleotides in length and sufficient to specifically hybridize under stringent conditions to PCIP mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

A preferred agent for detecting PCIP protein is an antibody capable of binding to  
5 PCIP protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or F(ab')<sub>2</sub>) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as  
10 indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated  
15 from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect PCIP mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of PCIP mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of PCIP protein include enzyme linked  
20 immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of PCIP genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of PCIP protein include introducing into a subject a labeled anti-PCIP antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a  
25 subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a serum sample or cerebrospinal fluid isolated by conventional means from a  
30 subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a

compound or agent capable of detecting PCIP protein, mRNA, or genomic DNA, such that the presence of PCIP protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of PCIP protein, mRNA or genomic DNA in the control sample with the presence of PCIP protein, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of PCIP in a biological sample. For example, the kit can comprise a labeled compound or agent capable of detecting PCIP protein or mRNA in a biological sample; means for determining the amount of PCIP in the sample; and means for comparing the amount of PCIP in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect PCIP protein or nucleic acid.

## 2. Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant PCIP expression or activity. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with a misregulation in PCIP protein activity or nucleic acid expression, such as a neurodegenerative disorder, *e.g.*, Alzheimer's disease, dementias related to Alzheimer's disease (such as Pick's disease), Parkinson's and other Lewy diffuse body diseases, multiple sclerosis, amyotrophic lateral sclerosis, progressive supranuclear palsy, epilepsy, spinocerebellar ataxia, and Jakob-Creutzfeldt disease; a psychiatric disorder, *e.g.*, depression, schizophrenic disorders, Korsakoff's psychosis, mania, anxiety disorders, bipolar affective disorders, or phobic disorders; a learning or memory disorder, *e.g.*, amnesia or age-related memory loss; a neurological disorder, *e.g.*, migraine; a pain disorder, *e.g.*, hyperalgesia or pain associated with musculoskeletal disorders; spinal cord injury; stroke; and head trauma; or a cardiovascular disorder, *e.g.*, sinus node dysfunction, angina, heart failure, hypertension, atrial fibrillation, atrial flutter, dilated cardiomyopathy, idiopathic cardiomyopathy, myocardial infarction, coronary artery disease, coronary artery spasm, or arrhythmia.

Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a disorder associated with a misregulation in PCIP protein activity or nucleic acid expression, such as a potassium channel associated disorder. Thus, the present invention provides a method for identifying a disease or disorder associated with  
5 aberrant PCIP expression or activity in which a test sample is obtained from a subject and PCIP protein or nucleic acid (*e.g.*, mRNA or genomic DNA) is detected, wherein the presence of PCIP protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant PCIP expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of  
10 interest. For example, a test sample can be a biological fluid (*e.g.*, serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate)  
15 to treat a disease or disorder associated with aberrant PCIP expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a CNS disorder or a cardiovascular disorder. Thus, the present invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant PCIP expression or activity in  
20 which a test sample is obtained and PCIP protein or nucleic acid expression or activity is detected (*e.g.*, wherein the abundance of PCIP protein or nucleic acid expression or activity is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant PCIP expression or activity).

The methods of the invention can also be used to detect genetic alterations in a  
25 PCIP gene, thereby determining if a subject with the altered gene is at risk for a disorder characterized by misregulation in PCIP protein activity or nucleic acid expression, such as a CNS disorder or a cardiovascular disorder. In preferred embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic alteration characterized by at least one of an alteration affecting the integrity of a  
30 gene encoding a PCIP-protein, or the mis-expression of the PCIP gene. For example, such genetic alterations can be detected by ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from a PCIP gene; 2) an addition of one or more

nucleotides to a PCIP gene; 3) a substitution of one or more nucleotides of a PCIP gene, 4) a chromosomal rearrangement of a PCIP gene; 5) an alteration in the level of a messenger RNA transcript of a PCIP gene, 6) aberrant modification of a PCIP gene, such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a PCIP gene, 8) a non-wild type level of a PCIP-protein, 9) allelic loss of a PCIP gene, and 10) inappropriate post-translational modification of a PCIP-protein. As described herein, there are a large number of assays known in the art which can be used for detecting alterations in a PCIP gene. A preferred biological sample is a tissue or serum sample isolated by conventional means from a subject.

In certain embodiments, detection of the alteration involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, *e.g.*, U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, *e.g.*, Landegran *et al.* (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:360-364), the latter of which can be particularly useful for detecting point mutations in the PCIP-gene (see Abravaya *et al.* (1995) *Nucleic Acids Res.* 23:675-682). This method can include the steps of collecting a sample of cells from a subject, isolating nucleic acid (*e.g.*, genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a PCIP gene under conditions such that hybridization and amplification of the PCIP-gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli, J.C. *et al.*, (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh, D.Y. *et al.*, (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi, P.M. *et al.* (1988) *Bio-Technology* 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection

schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in a PCIP gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example,  
5 sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score  
10 for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in PCIP can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotides probes (Cronin, M.T. *et al.*  
15 (1996) *Human Mutation* 7: 244-255; Kozal, M.J. *et al.* (1996) *Nature Medicine* 2: 753-759). For example, genetic mutations in PCIP can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, M.T. *et al. supra*. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by  
20 making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other  
25 complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the PCIP gene and detect mutations by comparing the sequence of the sample PCIP with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques  
30 developed by Maxam and Gilbert ((1977) *Proc. Natl. Acad. Sci. USA* 74:560) or Sanger ((1977) *Proc. Natl. Acad. Sci. USA* 74:5463). It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the



diagnostic assays ((1995) *Biotechniques* 19:448), including sequencing by mass spectrometry (see, *e.g.*, PCT International Publication No. WO 94/16101; Cohen *et al.* (1996) *Adv. Chromatogr.* 36:127-162; and Griffin *et al.* (1993) *Appl. Biochem. Biotechnol.* 38:147-159).

- 5           Other methods for detecting mutations in the PCIP gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242). In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type PCIP sequence with
- 10   potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digesting the mismatched regions. In
- 15   other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, for example, Cotton *et al.* (1988) *Proc. Natl Acad Sci USA* 85:4397; Saleeba *et al.*
- 20   (1992) *Methods Enzymol.* 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

- In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point
- 25   mutations in PCIP cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based on a PCIP sequence, *e.g.*, a wild-type PCIP sequence, is hybridized to a cDNA or other DNA
- 30   product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, for example, U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in PCIP genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl. Acad. Sci USA*: 86:2766, see also Cotton (1993) *Mutat. Res.* 285:125-144; and Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control PCIP nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change.

10 The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends*

15 *Genet* 7:5).

In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not

20 completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

Examples of other techniques for detecting point mutations include, but are not

25 limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions which permit hybridization only if a perfect match is found (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc. Natl Acad. Sci USA* 86:6230). Such allele specific

30 oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention.

Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving a PCIP gene.

Furthermore, any cell type or tissue in which PCIP is expressed may be utilized in the prognostic assays described herein.

### 3. Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (*e.g.*, drugs) on the expression or activity of a PCIP protein (*e.g.*, the modulation of membrane excitability or resting potential) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase PCIP gene expression, protein levels, or upregulate PCIP activity, can be monitored in clinical trials of subjects exhibiting decreased PCIP gene expression, protein levels, or downregulated PCIP activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease PCIP gene expression, protein levels, or downregulate PCIP activity, can be monitored in clinical trials of subjects exhibiting increased PCIP

gene expression, protein levels, or upregulated PCIP activity. In such clinical trials, the expression or activity of a PCIP gene, and preferably, other genes that have been implicated in, for example, a potassium channel associated disorder can be used as a "read out" or markers of the phenotype of a particular cell.

5           For example, and not by way of limitation, genes, including PCIP, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) which modulates PCIP activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on potassium channel associated disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and  
10           analyzed for the levels of expression of PCIP and other genes implicated in the potassium channel associated disorder, respectively. The levels of gene expression (*e.g.*, a gene expression pattern) can be quantified by northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of PCIP or  
15           other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during treatment of the individual with the agent.

          In a preferred embodiment, the present invention provides a method for  
20           monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) including the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of a PCIP protein, mRNA, or genomic DNA  
25           in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the PCIP protein, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the PCIP protein, mRNA, or genomic DNA in the pre-administration sample with the PCIP protein, mRNA, or genomic DNA in the post  
30           administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of PCIP to higher levels than detected,

*i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of PCIP to lower levels than detected, *i.e.* to decrease the effectiveness of the agent. According to such an embodiment, PCIP expression or activity may be used as an indicator of the effectiveness of an agent, even in the absence of an observable phenotypic response.

#### D. Methods of Treatment:

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant PCIP expression or activity. With regards to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics.

"Pharmacogenomics", as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers the study of how a patient's genes determine his or her response to a drug (*e.g.*, a patient's "drug response phenotype", or "drug response genotype".) Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic treatment with either the PCIP molecules of the present invention or PCIP modulators according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

##### 1. Prophylactic Methods

In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant PCIP expression or activity, by administering to the subject a PCIP or an agent which modulates PCIP expression or at least one PCIP activity. Subjects at risk for a disease which is caused or contributed to by aberrant PCIP expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the

PCIP aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of PCIP aberrancy, for example, a PCIP, PCIP agonist or PCIP antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

5

## 2. Therapeutic Methods

Another aspect of the invention pertains to methods of modulating PCIP expression or activity for therapeutic purposes. Accordingly, in an exemplary embodiment, the modulatory method of the invention involves contacting a cell with a  
10 PCIP or agent that modulates one or more of the activities of PCIP protein activity associated with the cell. An agent that modulates PCIP protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring target molecule of a PCIP protein (*e.g.*, a PCIP substrate), a PCIP antibody, a PCIP agonist or antagonist, a peptidomimetic of a PCIP agonist or antagonist, or other small molecule.  
15 In one embodiment, the agent stimulates one or more PCIP activities. Examples of such stimulatory agents include active PCIP protein and a nucleic acid molecule encoding PCIP that has been introduced into the cell. In another embodiment, the agent inhibits one or more PCIP activities. Examples of such inhibitory agents include antisense PCIP nucleic acid molecules, anti-PCIP antibodies, and PCIP inhibitors. These modulatory  
20 methods can be performed *in vitro* (*e.g.*, by culturing the cell with the agent) or, alternatively, *in vivo* (*e.g.*, by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of a PCIP protein or nucleic acid molecule. Examples of such disorders include CNS disorders such as neurodegenerative  
25 disorders, *e.g.*, Alzheimer's disease, dementias related to Alzheimer's disease (such as Pick's disease), Parkinson's and other Lewy diffuse body diseases, multiple sclerosis, amyotrophic lateral sclerosis, progressive supranuclear palsy, epilepsy, and Jakob-Creutzfeldt disease; psychiatric disorders, *e.g.*, depression, schizophrenic disorders, Korsakoff's psychosis, mania, anxiety disorders, bipolar affective disorders, or phobic  
30 disorders; learning or memory disorders, *e.g.*, amnesia or age-related memory loss; neurological disorders, *e.g.*, migraine; pain disorders, *e.g.*, hyperalgesia or pain associated with musculoskeletal disorders; spinal cord injury; stroke; and head trauma;

or cardiovascular disorders, *e.g.*, arteriosclerosis, ischemia reperfusion injury, restenosis, arterial inflammation, vascular wall remodeling, ventricular remodeling, rapid ventricular pacing, coronary microembolism, tachycardia, bradycardia, pressure overload, aortic bending, coronary artery ligation, vascular heart disease, atrial fibrillation, long-QT syndrome, congestive heart failure, sinus node dysfunction, angina, heart failure, hypertension, atrial fibrillation, atrial flutter, dilated cardiomyopathy, idiopathic cardiomyopathy, myocardial infarction, coronary artery disease, coronary artery spasm, or arrhythmia. In one embodiment, the method involves administering an agent (*e.g.*, an agent identified by a screening assay described herein), or combination of agents that modulates (*e.g.*, upregulates or downregulates) PCIP expression or activity. In another embodiment, the method involves administering a PCIP protein or nucleic acid molecule as therapy to compensate for reduced or aberrant PCIP expression or activity.

A preferred embodiment of the present invention involves a method for treatment of a PCIP associated disease or disorder which includes the step of administering a therapeutically effective amount of a PCIP antibody to a subject. As defined herein, a therapeutically effective amount of antibody (*i.e.*, an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of an antibody can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with antibody in the range of between about 0.1 to 20 mg/kg body weight, one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of antibody used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result from the results of diagnostic assays as described herein.

Stimulation of PCIP activity is desirable in situations in which PCIP is abnormally downregulated and/or in which increased PCIP activity is likely to have a beneficial effect. For example, stimulation of PCIP activity is desirable in situations in which a PCIP is downregulated and/or in which increased PCIP activity is likely to have a beneficial effect. Likewise, inhibition of PCIP activity is desirable in situations in which PCIP is abnormally upregulated and/or in which decreased PCIP activity is likely to have a beneficial effect.

### 3. Pharmacogenomics

The PCIP molecules of the present invention, as well as agents, or modulators which have a stimulatory or inhibitory effect on PCIP activity (*e.g.*, PCIP gene expression) as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) potassium channel associated disorders associated with aberrant PCIP activity (*e.g.*, CNS disorders such as neurodegenerative disorders, *e.g.*, Alzheimer's disease, dementias related to Alzheimer's disease (such as Pick's disease), Parkinson's and other Lewy diffuse body diseases, multiple sclerosis, amyotrophic lateral sclerosis, progressive supranuclear palsy, epilepsy, spinocerebellar ataxia, and Jakob-Creutzfeldt disease; psychiatric disorders, *e.g.*, depression, schizophrenic disorders, Korsakoff's psychosis, mania, anxiety disorders, bipolar affective disorders, or phobic disorders; learning or memory disorders, *e.g.*, amnesia or age-related memory loss; neurological disorders, *e.g.*, migraine; pain disorders, *e.g.*, hyperalgesia or pain associated with musculoskeletal disorders; spinal cord injury; stroke; and head trauma; or cardiovascular disorders such as arteriosclerosis, ischemia reperfusion injury, restenosis, arterial inflammation, vascular wall remodeling, ventricular remodeling, rapid ventricular pacing, coronary microembolism, tachycardia, bradycardia, pressure overload, aortic bending, coronary artery ligation, vascular heart disease, atrial fibrillation, long-QT syndrome, congestive heart failure, sinus node dysfunction, angina, heart failure, hypertension, atrial fibrillation, atrial flutter, dilated cardiomyopathy, idiopathic cardiomyopathy, myocardial infarction, coronary artery disease, coronary artery spasm, or arrhythmia). In conjunction with such treatment, pharmacogenomics (*i.e.*, the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) may be considered.



Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to  
5 administer a PCIP molecule or PCIP modulator as well as tailoring the dosage and/or therapeutic regimen of treatment with a PCIP molecule or PCIP modulator.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, for example, Eichelbaum, M. *et al.* (1996) *Clin. Exp. Pharmacol. Physiol.*  
10 23(10-11) :983-985 and Linder, M.W. *et al.* (1997) *Clin. Chem.* 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur  
15 either as rare genetic defects or as naturally-occurring polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

20 One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (*e.g.*, a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants.) Such a high-resolution  
25 genetic map can be compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be generated from a combination of some ten-million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a  
30 "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-

associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner, treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among  
5 such genetically similar individuals.

Alternatively, a method termed the "candidate gene approach", can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drug's target is known (*e.g.*, a PCIP protein of the present invention), all common variants of that gene can be fairly easily identified in the population and it can  
10 be determined if having one version of the gene versus another is associated with a particular drug response.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT  
15 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among  
20 different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as  
25 demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Alternatively, a method termed the "gene expression profiling", can be utilized to  
30 identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (*e.g.*, a PCIP molecule or PCIP modulator of the present

invention) can give an indication whether gene pathways related to toxicity have been turned on.

Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a PCIP molecule or PCIP modulator, such as a modulator identified by one of the exemplary screening assays described herein.

#### 4. Use of PCIP Molecules as Surrogate Markers

The PCIP molecules of the invention are also useful as markers of disorders or disease states, as markers for precursors of disease states, as markers for predisposition of disease states, as markers of drug activity, or as markers of the pharmacogenomic profile of a subject. Using the methods described herein, the presence, absence and/or quantity of the PCIP molecules of the invention may be detected, and may be correlated with one or more biological states *in vivo*. For example, the PCIP molecules of the invention may serve as surrogate markers for one or more disorders or disease states or for conditions leading up to disease states.

As used herein, a "surrogate marker" is an objective biochemical marker which correlates with the absence or presence of a disease or disorder, or with the progression of a disease or disorder (*e.g.*, with the presence or absence of a tumor). The presence or quantity of such markers is independent of the causation of the disease. Therefore, these markers may serve to indicate whether a particular course of treatment is effective in lessening a disease state or disorder. Surrogate markers are of particular use when the presence or extent of a disease state or disorder is difficult to assess through standard methodologies (*e.g.*, early stage tumors), or when an assessment of disease progression is desired before a potentially dangerous clinical endpoint is reached (*e.g.*, an assessment of cardiovascular disease may be made using cholesterol levels as a surrogate marker, and an analysis of HIV infection may be made using HIV RNA levels as a surrogate marker, well in advance of the undesirable clinical outcomes of myocardial infarction or fully-developed AIDS). Examples of the use of surrogate markers in the art include: Koomen *et al.* (2000) *J. Mass. Spectrom.* 35:258-264; and James (1994) *AIDS Treatment News Archive* 209.

The PCIP molecules of the invention are also useful as pharmacodynamic markers. As used herein, a "pharmacodynamic marker" is an objective biochemical marker which correlates specifically with drug effects. The presence or quantity of a pharmacodynamic marker is not related to the disease state or disorder for which the drug is being administered; therefore, the presence or quantity of the marker is indicative of the presence or activity of the drug in a subject. For example, a pharmacodynamic marker may be indicative of the concentration of the drug in a biological tissue, in that the marker is either expressed or transcribed or not expressed or transcribed in that tissue in relationship to the level of the drug. In this fashion, the distribution or uptake of the drug may be monitored by the pharmacodynamic marker. Similarly, the presence or quantity of the pharmacodynamic marker may be related to the presence or quantity of the metabolic product of a drug, such that the presence or quantity of the marker is indicative of the relative breakdown rate of the drug *in vivo*. Pharmacodynamic markers are of particular use in increasing the sensitivity of detection of drug effects, particularly when the drug is administered in low doses. Since even a small amount of a drug may be sufficient to activate multiple rounds of marker (*e.g.*, a PCIP marker) transcription or expression, the amplified marker may be in a quantity which is more readily detectable than the drug itself. Also, the marker may be more easily detected due to the nature of the marker itself; for example, using the methods described herein, anti-PCIP antibodies may be employed in an immune-based detection system for a PCIP protein marker, or PCIP-specific radiolabeled probes may be used to detect a PCIP mRNA marker. Furthermore, the use of a pharmacodynamic marker may offer mechanism-based prediction of risk due to drug treatment beyond the range of possible direct observations. Examples of the use of pharmacodynamic markers in the art include: Matsuda *et al.* US 6,033,862; Hattis *et al.* (1991) *Env. Health Perspect.* 90:229-238; Schentag (1999) *Am. J. Health-Syst. Pharm.* 56 Suppl. 3:S21-S24; and Nicolau (1999) *Am. J. Health-Syst. Pharm.* 56 Suppl. 3:S16-S20.

The PCIP molecules of the invention are also useful as pharmacogenomic markers. As used herein, a "pharmacogenomic marker" is an objective biochemical marker which correlates with a specific clinical drug response or susceptibility in a subject (see, *e.g.*, McLeod *et al.* (1999) *Eur. J. Cancer* 35(12):1650-1652). The presence or quantity of the pharmacogenomic marker is related to the predicted response of the subject to a specific drug or class of drugs prior to administration of the drug. By assessing the presence or quantity of one or more pharmacogenomic markers in a subject, a drug therapy which is most appropriate for the subject, or which is predicted to have a greater degree of success, may be selected. For example, based on the presence or quantity of RNA, or protein (*e.g.*, PCIP protein or RNA) for specific tumor markers in a subject, a drug or course of treatment may be selected that is optimized for the

treatment of the specific tumor likely to be present in the subject. Similarly, the presence or absence of a specific sequence mutation in PCIP DNA may correlate PCIP drug response. The use of pharmacogenomic markers therefore permits the application of the most appropriate treatment for each subject without having to administer the  
5 therapy.

This invention is further illustrated by the following examples, which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application, as well as the Figures and the Sequence  
10 Listing are incorporated herein by reference.

### EXAMPLES

The following materials and methods were used in the Examples.

#### 15 Strains, plasmids, bait cDNAs, and general microbiological techniques

Basic yeast strains (HF7c, Y187,) bait (pGBT9) and fish (pACT2) plasmids used in this work were purchased from Clontech (Palo Alto, CA). cDNAs encoding rat Kv4.3, Kv4.2, and Kv1.1, were provided by Wyeth-Ayerst Research (865 Ridge Rd., Monmouth Junction, NJ 08852) Standard yeast media including synthetic complete  
20 medium lacking L-leucine, L-tryptophan, and L-histidine were prepared and yeast genetic manipulations were performed as described (Sherman (1991) *Meth. Enzymol.* 194:3-21). Yeast transformations were performed using standard protocols (Gietz *et al.* (1992) *Nucleic Acids Res.* 20:1425; Ito *et al* (1983) *J. Bacteriol.* 153:163-168). Plasmid DNAs were isolated from yeast strains by a standard method (Hoffman and Winston  
25 (1987) *Gene* 57:267-272).

#### Bait and Yeast Strain Construction

The first 180 amino acids of rKv4.3 (described in Serdio P. *et al.* (1996) *J. Neurophys* 75:2174-2179) were amplified by PCR and cloned in frame into pGBT9  
30 resulting in plasmid pFWA2, (hereinafter "bait"). This bait was transformed into the two-hybrid screening strain HF7c and tested for expression and self-activation. The bait was validated for expression by Western blotting. The rKv4.3 bait did not self-activate in the presence of 10 mM 3-amino-1,2,3-Triazole (3-AT).

### Library construction

Rat mid brain tissue was provided by Wyeth-Ayerst Research (Monmouth Junction, NJ). Total cellular RNA was extracted from the tissues using standard techniques (Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, (1989)). mRNA was prepared using a Poly-A Spin mRNA Isolation Kit from New England Biolabs (Beverly, MA). cDNA from the mRNA sample was synthesized using a cDNA Synthesis Kit from Stratagene (La Jolla, CA) and ligated into pACT2's EcoRI and XhoI sites, giving rise to a two-hybrid library.

### Two-Hybrid Screening

Two-hybrid screens were carried out essentially as described in Bartel, P. *et al.* (1993) "Using the Two-Hybrid System to Detect Polypeptide-Polypeptide Interactions" in *Cellular Interactions in Development: A Practical Approach*, Hartley, D.A. ed. Oxford University Press, Oxford, pp. 153-179, with a bait-library pair of rkv4.3 bait-rat mid brain library. A filter disk beta-galactosidase (beta-gal) assay was performed essentially as previously described (Brill *et al.* (1994) *Mol. Biol. Cell.* 5:297-312). Clones that were positive for both reporter gene activity (His and beta-galactosidase) were scored and fish, plasmids were isolated from yeast, transformed into *E. coli* strain KC8, DNA plasmids were purified and the resulting plasmids were sequenced by conventional methods (Sanger F. *et al.* (1977) *PNAS*, 74: 5463-67).

### Specificity test

Positive interactor clones were subjected to a binding specificity test where they were exposed to a panel of related and unrelated baits by a mating scheme previously described (Finley R.L. Jr. *et al.* (1994) *PNAS*, 91(26):12980-12984). Briefly, positive fish plasmids were transformed into Y187 and the panel of baits were transformed into HF7c. Transformed fish and bait cells were streaked out as stripes on selective medium plates, mated on YPAD plates, and tested for reporter gene activity.

### Analysis

PCIP nucleotides were analyzed for nucleic acid hits by the BLASTN 1.4.8MP program (Altschul *et al.* (1990) Basic Local Alignment Search Tool. *J. Mol. Biol.* 215: 403-410). PCIP proteins were analyzed for polypeptide hits by the BLASTP 1.4.9MP program.

### Electrophysiology methods

#### *Mammalian in vitro studies*

- HEK 293 and CHO cells were used for recordings 1–3 days after a transient transfection. Whole-cell currents were recorded from cells expressing GFP, identified by their green fluorescence. Electrodes pulled from filamented borosilicate glass (Sutter Instrument Co, Novato, CA) had an initial resistance of 3-5 MOhms. After Gigaseal and ruptured whole-cell configuration access, resistance was less than 10 MOhms. Whole-cell bath solutions were made from a 10X Hank's balanced salt solution (GibcoBRL) with the following final concentration (in mM): 138 NaCl, 5.4 KCl, 1.3 MgCl<sub>2</sub>, 1.3 CaCl<sub>2</sub>, 5.5 D-Glucose and 10 HEPES, pH 7.4. The intracellular electrode solution consisted of (in mM) 140 KCl, 10 HEPES, 10 EGTA, 0.5 MgCl<sub>2</sub>, pH 7.3. All chemicals were from Sigma (St. Louis, MO) or Fisher Scientific (Houston, TX).
- Membrane currents were recorded using a EPC9 patch-clamp amplifier (HEKA, Germany). Data were analyzed using Matlab (Natick, Ma), and leak subtracted if necessary. All experiments were done at room temperature.

#### *Xenopus oocyte studies*

- Frogs underwent no more than two surgeries and surgeries were performed by well established techniques. Frogs were anesthetized with ice. Total cRNA (1–10 ng) was injected into stage IV *Xenopus* oocytes that were harvested the previous day. The *Xenopus* oocytes were incubated in ND96 containing (in mM) 96 NaCl, 2 KCl, 1.8 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 5 HEPES, pH 7.6, plus (Gentamycin 50 µg/ml) at 18°C. *Xenopus* oocytes were studied 3–7 days after injection. The two-electrode voltage-clamp recordings were performed in ND96 solution using a TURBO TEC 03 Clamp Amplifier (ALA Scientific Instruments, Westbury, NY). Both electrodes were filled with 3 M KCl and had electrode resistances ranging from 0.2-1 MOhm. The current signals were filtered at 1000 Hz before transferred on a PC (Gateway, CA) using the PULSE software (HEKA, Germany).

**EXAMPLE 1: IDENTIFICATION OF RAT PCIP cDNAs**

The Kv4.3 gene coding sequence (coding for the first 180 amino acids) was amplified by PCR and cloned into pGBT9 creating a GAL4 DNA-binding domain-Kv4.3(1-180) gene fusion (plasmid pFWA2). HF7c was transformed with this construct. The resulting strain grew on synthetic complete medium lacking L-tryptophan but not on synthetic complete medium lacking L-tryptophan and L-histidine in the presence of 10mM 3-AT demonstrating that the {GAL4 DNA-binding domain}-{vKv4.3(1-180)} gene fusion does not have intrinsic transcriptional activation activity higher than the threshold allowed by 10mM 3-AT.

In this example, a yeast two-hybrid assay was performed in which a plasmid containing a {GAL4 DNA-binding domain}-{rKv4.3(1-180)} gene fusion was introduced into the yeast two-hybrid screening strain HF7c described above. HF7c was then transformed with the rat mid brain two-hybrid library. Approximately six million transformants were obtained and plated in selection medium. Colonies that grew in the selection medium and expressed the beta-galactosidase reporter gene were further characterized and subjected to retransformation and specificity assays. The retransformation and specificity tests yielded three PCIP clones (rat 1v, 8t, and 9qm) that were able to bind to the Kv4.3 polypeptide.

The full length sequences for the rat 1v gene, and partial sequences for 8t and 9q genes were derived as follows. The partial rat PCIP sequences were used to prepare probes, which were then used to screen, for example, rat mid brain cDNA libraries. Positive clones were identified, amplified and sequenced using standard techniques, to obtain the full length sequence. Additionally, a rapid amplification of the existing rat PCIP cDNA ends (using for example, 5' RACE, by Gibco, BRL) was used to complete the 5' end of the transcript.

**EXAMPLE 2: IDENTIFICATION OF HUMAN 1v cDNA**

To obtain the human 1v nucleic acid molecule, a cDNA library made from a human hippocampus (Clontech, Palo Alto, CA) was screened under low stringency conditions as follows: Prehybridization for 4 hours at 42°C in Clontech Express Hyb solution, followed by overnight hybridization at 42°C. The probe used was a PCR-generated fragment including nucleotides 49-711 of the rat sequence labeled with <sup>32</sup>P



dCTP. The filters were washed 6 times in 2XSSC/0.1% SDS at 55°C. The same conditions were used for secondary screening of the positive isolates. Clones thus obtained were sequenced using an ABI automated DNA Sequencing system, and compared to the rat sequences shown in SEQ ID NO:3 as well as to known sequences from the GenBank database. The largest clone from the library screen was subsequently subcloned into pBS-KS+ (Stratagene, La Jolla, CA) for sequence verification. The 515 base pair clone was determined to represent the human homolog of the 1v gene, encompassing 211 base pairs of 5' UTR and a 304 base pair coding region. To generate the full-length cDNA, 3' RACE was used according to the manufacturers instructions (Clontech Advantage PCR kit).

### **EXAMPLE 3: ISOLATION AND CHARACTERIZATION OF 1V SPLICE VARIANTS**

The mouse 1v shown in SEQ ID NO:5 and the rat 1vl splice variant shown in SEQ ID NO:7 was isolated using a two-hybrid assay as described in Example 1. The mouse 1vl splice variant shown in SEQ ID NO: 7 was isolated by screening a mouse brain cDNA library, and the rat 1vn splice variant shown in SEQ ID NO:11 was isolated by BLAST searching.

### **EXAMPLE 4: ISOLATION AND IDENTIFICATION OF 9Q AND OTHER PCIPs**

Rat 9ql (SEQ ID NO: 15) was isolated by database mining, rat 9qm (SEQ ID NO: 21) was isolated by a two-hybrid assay, and rat 9qc (SEQ ID NO:27) was identified by database mining. Human 9ql (SEQ ID NO: 13), and human 9qs (SEQ ID NO: 23) were identified as described in Example 2. Mouse 9ql (SEQ ID NO:17), monkey 9qs (SEQ ID NO:25), human p193 (SEQ ID NO:39), rat p19 (SEQ ID NO:33), and mouse p19 (SEQ ID NO:35) were identified by database mining. Rat 8t (SEQ ID NO:29) was identified using a two-hybrid assay. The sequence of W28559 (SEQ ID NO:37) was identified by database mining and sequencing of the identified EST with Genbank Accession Number AI352454. The protein sequence was found to contain a 41 amino acid region with strong homology to 1v, 9ql, and p19 (see alignment in Figure 25). However, downstream of this homologous region the sequence diverges from that

of the PCIP family. This sequence could represent a gene which possesses a 41 amino acid domain with homology to a similar domain found in the PCIP family members.

The human genomic 9q sequence (SEQ ID NOs:46 and 47) was isolated by screening a BAC genomic DNA library (Research Genetics) using primers which were  
5 designed based on the sequence of the human 9qm cDNA. Two positive clones were identified (448O2 and 721I17) and sequenced.

#### **EXAMPLE 5:        EXPRESSION OF 1V, 8T, AND 9Q mRNA IN RAT                              TISSUES**

10        Rat and mouse multiple tissue Northern blots (Clontech) were probed with a [32P]-labeled cDNA probe directed at the 5'-untranslated and 5'-coding region of the rat 1v sequence (nucleotides 35-124; SEQ ID NO:3) (this probe is specific for rat 1v and rat 1vl), the 5' coding region of the 8t sequence (nucleotides 1-88; SEQ ID NO:29) (this probe is specific for 8t), or the 5' end of the rat 9qm sequence (nucleotides 1-195; SEQ  
15 ID NO:21) (this probe is specific for all 9q isoforms, besides 8t). Blots were hybridize using standard techniques. Northern blots hybridized with the rat 1v probe revealed a single band at 2.3kb only in the lane containing brain RNA, suggesting that 1v expression is brain specific. Northern blots probed with the rat 8t probe revealed a major band at 2.4kb. The rat 8t band was most intense in the lane containing heart RNA and  
20 there was also a weaker band in the lane containing brain RNA. Northern blots hybridized with the 9q cDNA probe revealed a major band at 2.5kb and a minor band at over 4kb with predominant expression in brain and heart. The minor band may represent incompletely spliced or processed 9q mRNA. The results from the northern blots further indicated that p19 is expressed predominantly in the heart.

25

#### **EXAMPLE 6:        EXPRESSION OF 1V, 8T, AND 9Q IN BRAIN**

Expression of the rat 1v and 8t/9q genes in the brain was examined by *in situ* hybridization histochemistry (ISHH) using [35S]-labeled cRNA probes and a hybridization procedure identical to that described in Rhodes *et al.* (1996) J. Neurosci.,  
30 16:4846-4860. Templates for preparing the cRNA probes were generated by standard PCR methods. Briefly, oligonucleotide primers were designed to amplify a fragment of 3'- or 5'-untranslated region of the target cDNA and in addition, add the promoter

recognition sequences for T7 and T3 polymerase. Thus, to generate a 300 nucleotide probe directed at the 3'-untranslated region of the 1v mRNA, we used the following primers:

5-TAATACGACTCACTATAGGGACTGGCCATCCTGCTCTCAG-3 (T7, forward,

5 sense; SEQ ID NO:42)

5-ATTAACCCTCACTAAAGGGACACTACTGTTTAAGCTCAAG-3 (T3, reverse, antisense; SEQ ID NO:43). The underlined bases correspond to the T7 and T3 promoter sequences. To generate a probe directed at a 325 bp region of 3'-untranslated sequence shared by the 8t and 9q mRNAs, the following primers were used:

10 5-TAATACGACTCACTATAGGGCACCTCCCCTCCGGCTGTTC-3 (T7, forward, sense; SEQ ID NO:44)

5-ATTAACCCTCACTAAAGGGGAGAGCAGCAGCATGGCAGGGT-3 (T3, reverse, antisense; SEQ ID NO:45).

Autoradiograms of rat brain tissue sections processed for ISHH localization of 1v  
15 or 8t/9q mRNA expression revealed that 1v mRNA is expressed widely in brain in a pattern consistent with labeling of neurons as opposed to glial or endothelial cells. 1v mRNA is highly expressed in cortical, hippocampal, and striatal interneurons, the reticular nucleus of the thalamus, the medial habenula, and in cerebellar granule cells. 1v mRNA is expressed at moderate levels in midbrain nuclei including the substantia nigra  
20 and superior colliculus, in several other thalamic nuclei, and in the medial septal and diagonal band nuclei of the basal forebrain.

Because the probe used to analyze the expression of 8t and 9q hybridizes to a region of the 3'-untranslated region that is identical in the 8t and 9q mRNAs, this probe generates a composite image that reveals that 8t/9q mRNA is expressed widely in brain  
25 in a pattern that partly overlaps with that for 1v as described above. However, 8t/9q mRNA is highly expressed in the striatum, hippocampal formation, cerebellar granule cells, and neocortex. 8t/9q mRNA is expressed at moderate levels in the midbrain, thalamus, and brainstem. In many of these areas, 8t/9q mRNA appears to be concentrated in interneurons in addition to principal cells, and in all regions 8t/9q  
30 expression appears to be concentrated in neurons as opposed to glial cells.

Single- and double-label immunohistochemistry revealed that the PCIP and Kv4 polypeptides are precisely colocalized in many of the cell types and brain regions where PCIP and Kv4 mRNAs are coexpressed. For example, 9qm colocalized with Kv4.2 in the somata and dendrites of hippocampal granule and pyramidal cells, neurons in the medial habenular nucleus and in cerebellar basket cells, while 1v colocalized with Kv4.3 in layer II neurons of posterior cingulate cortex, hippocampal interneurons, and in a subset of cerebellar granule cells. Immunoprecipitation analyses indicated that 1v and 9qm are coassociated with Kv4  $\alpha$ -subunits in rat brain membranes.

#### 10    **EXAMPLE 7:            CO-ASSOCIATION OF PCIPs AND Kv4 CHANNELS** **IN COS AND CHO CELLS**

COS1 and CHO cells were transiently transfected with individual PCIPs (KChIP1, KChIP2, KChIP3) alone or together with Kv4.2 or Kv4.3 using the lipofectamine plus procedure essentially as described by the manufacturer (Boehringer Mannheim). Forty-eight hours after the transfection, cells were washed, fixed, and processed for immunofluorescent visualization as described previously (Bekele-Arcuri *et al.* (1996) *Neuropharmacology*, 35:851-865). Affinity-purified rabbit polyclonal or mouse monoclonal antibodies to the Kv4 channel or the PCIP protein were used for immunofluorescent detection of the target proteins.

When expressed alone, the PCIPs were diffusely distributed throughout the cytoplasm of COS-1 and CHO cells, as would be expected for cytoplasmic proteins. In contrast, when expressed alone, the Kv4.2 and Kv4.3 polypeptides were concentrated within the perinuclear ER and Golgi compartments, with some immunoreactivity concentrated in the outer margins of the cell. When the PCIPs were coexpressed with Kv4  $\alpha$ -subunits, the characteristic diffuse PCIP distribution changed dramatically, such that the PCIPs precisely colocalized with the Kv4  $\alpha$ -subunits. This redistribution of the PCIPs did not occur when they were coexpressed with the Kv1.4  $\alpha$ -subunit, indicating that altered PCIP localization is not a consequence of overexpression and that these PCIPs associate specifically with Kv4-family  $\alpha$ -subunits.

To verify that the PCIP and Kv4 polypeptides are tightly associated and not simply colocalized in co-transfected cells, reciprocal immunoprecipitation analyses were performed using the PCIP and channel-specific antibodies described above. All three

PCIP polypeptides coassociated with Kv4  $\alpha$ -subunits in cotransfected cells, as evidenced by the ability of anti-Kv4.2 and anti-Kv4.3 antibodies to immunoprecipitate the KChIP1, KChIP2, and KChIP3 proteins from lysates prepared from cotransfected cells, and by the ability of anti-PCIP antibodies to immunoprecipitate Kv4.2 and Kv4.3  $\alpha$ -subunits from these same lysates. The cells were lysed in buffer containing detergent and protease inhibitors, and prepared for immunoprecipitation reactions essentially as described previously (Nakahira *et al.* (1996) *J. Biol. Chem.*, 271:7084-7089).

Immunoprecipitations were performed as described in Nakahira *et al.* (1996) *J. Biol. Chem.*, 271:7084-7089 and in Harlow E. and Lane, D., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, c1988. The products resulting from the immunoprecipitation were size fractionated by SDS-PAGE and transferred to nitrocellulose filters using standard procedures.

To confirm that the cytoplasmic N-terminus of Kv4 channels is sufficient for the interaction with the PCIPs KChIP1 or KChIP2 were co-expressed with a Kv4.3 mutant (Kv4.3 $\Delta$ C) that lacks the entire 219 amino acid cytoplasmic C-terminal tail. In transiently transfected COS-1 cells, the Kv4.3 $\Delta$ C mutant was extensively trapped within the perinuclear ER and Golgi: little or no staining was observed at the outer margins of the cell. Nonetheless, KChIP1 and KChIP2 precisely colocalized with Kv4.3 $\Delta$ C in cotransfected cells, and moreover, Kv4.3 $\Delta$ C was efficiently coimmunoprecipitated by PCIP antibodies, indicating that the interaction of these PCIPs with Kv4  $\alpha$ -subunits does not require the cytoplasmic C-terminus of the channel.

#### **EXAMPLE 8: CO-ASSOCIATION OF PCIPs AND Kv4 CHANNELS IN NATIVE TISSUES**

To determine whether PCIPs colocalize and co-associate with Kv4 subunits in native tissues, Kv4- and PCIP-specific antibodies were used for single and double-label immunohistochemical analyses and for reciprocal coimmunoprecipitation analyses of rat brain membranes. Immunohistochemical staining of rat brain sections indicated that KChIP1 and KChIP2 colocalize with Kv4.2 and Kv4.3 in a region and cell type-specific manner. For example, KChIP1 colocalized with Kv4.3 in hippocampal interneurons, cerebellar granule cells, and cerebellar glomeruli, a specialized synaptic arrangement

between the dendrites of cerebellar basket and golgi cells and mossy fiber terminals. KChIP2 colocalized with Kv4.3 and Kv4.2 in the dendrites of granule cells in the dentate gyrus, in the apical and basal dendrites of hippocampal and neocortical pyramidal cells, and in several subcortical structures including the striatum and superior colliculus. Co-immunoprecipitation analyses performed using synaptic membranes prepared from whole rat brain revealed that the PCIPs (KChIPs 1, 2, and 3) are tightly associated with Kv4.2 and Kv4.3 in brain K<sup>+</sup> channel complexes. Anti-PCIP antibodies immunoprecipitated Kv4.2 and Kv4.3 from brain membranes, and anti-Kv4.2 and Kv4.3 antibodies immunoprecipitated the PCIPs. None of the PCIP polypeptides were immunoprecipitated by anti-Kv2.1 antibodies, indicating that the association of these PCIPs with brain Kv channels may be specific for Kv4  $\alpha$ -subunits. Taken together, these anatomical and biochemical analyses indicate that these PCIPs are integral components of native Kv4 channel complexes.

#### 15 **EXAMPLE 9: PCIPs ARE CALCIUM BINDING PROTEINS**

To determine whether KChIPs 1, 2, and 3 bind Ca<sup>2+</sup>, GST-fusion proteins were generated for each PCIP and the ability of the GST-PCIP proteins, as well as the recombinant PCIP polypeptides enzymatically cleaved from GST, to bind <sup>45</sup>Ca<sup>2+</sup> was examined using a filter overlay assay (described in, for example, Kobayashi *et al.* (1993) Biochem. Biophys. Res. Commun. 189(1):511-7). All three PCIP polypeptides, but not an unrelated GST-fusion protein, display strong <sup>45</sup>Ca<sup>2+</sup> binding in this assay. Moreover, all three PCIP polypeptides display a Ca<sup>2+</sup>-dependent mobility shift on SDS-PAGE, indicating that like the other members of this family, KChIPs 1, 2 and 3 are in fact Ca<sup>2+</sup>-binding proteins (Kobayashi *et al.* (1993) *supra*; Buxbaum *et al.* Nef (1996). Neuron-specific calcium sensors (the NCS-1 subfamily). In: Celio MR (ed) Guidebook to the calcium-binding proteins. Oxford University Press, New York, pp94-98; Buxbaum J.D., *et al.* (1998) *Nature Med.* 4(10):1177-81.

#### 30 **EXAMPLE 10: ELECTROPHYSIOLOGICAL CHARACTERIZATION OF PCIPs**

Because PCIPs, *e.g.*, KChIP1 (1v), KChIP2 (9ql), and KChIP3 (p19), colocalize and coassociate with Kv4  $\alpha$ -subunits in brain, another critical question was to determine

whether these PCIPs alter the conductance properties of Kv4 channels. To address this issue, Kv4.2 and Kv4.3 were expressed alone and in combination with individual PCIPs. CHO cells were transiently-transfected with cDNA using the DOTAP lipofection method as described by the manufacturer (Boehringer Mannheim, Inc.). Transfected cells were identified by cotransfecting enhanced GFP along with the genes of interest and subsequently determining if the cells contained green GFP fluorescence. Currents in CHO cells were measured using the patch-clamp technique (Hamill *et al.* 1981. Pfluegers Arch. 391: 85-100).

Transient transfection of the rat Kv4.2  $\alpha$ -subunit in CHO cells resulted in expression of a typical A-type K<sup>+</sup> conductance. Coexpression of Kv4.2 with KChIP1 revealed several dramatic effects of KChIP1 on the channel (Figure 41 and Table 1). First, the amplitude of the Kv4.2 current increased approximately 7.5 fold in the presence of KChIP1 (amplitude of Kv4.2 alone = 0.60 +/- 0.096 nA/cell; Kv4.2 + KChIP1 = 4.5 +/- 0.55 nA/cell). When converted into current density by correcting for cell capacitance, a measure of cell surface membrane area, the Kv4.2 current density increased 12 fold with coexpression of KChIP1 (Kv4.2 alone = 25.5 +/- 3.2 pA/pF; Kv4.2 + KChIP1 = 306.9 +/- 57.9 pA/pF), indicating that KChIPs promote and/or stabilize Kv4.2 surface expression. Together with this increase in current density, a dramatic leftward shift in the threshold for activation of Kv4.2 currents was observed in cells expressing Kv4.2 and KChIP1 (activation V<sub>1/2</sub> for Kv4.2 alone = 20.8 +/- 7.0mV, Kv4.2 + KChIP1 = -12.1 +/- 1.4 mV). Finally, the kinetics of Kv4.2 inactivation slowed considerably when Kv4.2 was coexpressed with KChIP1 (inactivation time constant of Kv4.2 alone = 28.2 +/- 2.6 ms; Kv4.2 + KChIP1 = 104.1 +/- 10.4 ms), while channels recovered from inactivation much more rapidly in cells expressing both Kv4.2 and KChIP1 (recovery tau = 53.6 +/- 7.6 ms) versus cells expressing Kv4.2 alone (recovery tau = 272.2 +/- 26.1 ms).

KChIPs1, 2 and 3 have distinct N-termini but share considerable amino acid identity within the C-terminal "core" domain. Despite their distinct N-termini, the effects of KChIP2 and KChIP3 on Kv4.2 current density and kinetics were strikingly similar to those produced by KChIP1 (Table1). Thus to confirm that the conserved C-terminal core domain, which contains all three EF-hands, is sufficient to modulate Kv4 current density and kinetics, N-terminal truncation mutants of KChIP1 and KChIP2

were prepared. The KChIP1 $\Delta$ N2-31 and KChIP2 $\Delta$ N2-67 mutants truncated KChIP1 and KChIP2, respectively, to the C-terminal 185 amino acid core sequence.

Coexpression of KChIP1 $\Delta$ N2-31 or KChIP2 $\Delta$ N2-67 with Kv4.2 in CHO cells produced changes in Kv4.2 current density and kinetics that were indistinguishable from the

5 effects produced by full-length KChIP1 or KChIP2 (Table1).

To investigate whether the modulatory effects of these KChIPs are specific for Kv4 channels, KChIP1 was coexpressed with Kv1.4 and Kv2.1 in *Xenopus* oocytes. *Xenopus* oocytes were injected with 1-3 ng/oocyte of cRNA which was prepared using standard in vitro transcription techniques (Sambrook *et al.* 1989. Molecular Cloning: a  
10 laboratory manual, Cold Spring Harbor Press). Currents in *Xenopus* oocytes were measured with a two-electrode voltage clamp. KChIP1 did not appear to have any effect on Kv1.4 or Kv2.1 currents (Table2), indicating that these functional effects may be specific for Kv4 channels. As a final control for the KChIP effects and to verify that the KChIPs' effects on Kv4 currents are independent of expression system, the above kinetic  
15 analyses were repeated after expressing Kv4.3 and KChIP mRNAs in *Xenopus* oocytes. The effects KChIP1 on for Kv4.3 in the *Xenopus* oocyte system were strikingly similar to those on Kv4.2 in CHO cells (Table1).

Since these KChIPs bind Ca<sup>2+</sup>, another important question is to determine whether the effects of KChIP1 on Kv4.2 currents are Ca<sup>2+</sup>-dependent. This question  
20 was addressed indirectly by introducing point mutations within each of KChIP1's EF-hand domains: one mutant has point mutations in the first two EF hands (D<sub>199</sub> to A, G<sub>104</sub> to A, D<sub>135</sub> to A, and G<sub>140</sub> to A) and the other one has point mutations in all three EF hands (D<sub>199</sub> to A, G<sub>104</sub> to A, D<sub>135</sub> to A, G<sub>140</sub> to A, D<sub>183</sub> to A, and G<sub>188</sub> to A). These mutations substituted alanine for the two most highly conserved amino acids within the EF-hand  
25 consensus (Figure 25; Linse, S. and Forsen, S. (1995) Determinants that govern high-affinity Calcium binding. In Means, S. (Ed.) Advances in second messenger and phosphoprotein research. New York, Ravens Press,. 30:89-150). Coexpression of this KChIP1 triple EF-hand mutant with Kv4.2 or Kv4.3 in COS cells indicated that this mutant colocalizes and is efficiently coimmunoprecipitated with Kv4  $\alpha$ -subunits in  
30 COS-1 cells. However, these EF-hand point mutations completely eliminated the effects of KChIP1 on Kv4.2 kinetics (Table 1). Taken together, these results indicate that the binding interaction between KChIP1 and Kv4.2 is Ca<sup>2+</sup> independent, while modulation



of Kv4.2 kinetics by KChIP1 is either  $\text{Ca}^{2+}$ -dependent or sensitive to structural changes induced by point mutations within the EF-hand domains.

**TABLE 1****5 Functional effect of KChIPs on Kv4 channels**

Current Parameter	rKv4.2 + vector	rKv4.2 + KChIP1	rKv4.2 + KChIP1 $\Delta\text{N2-31}$	rKv4.2 + KChIP2	rKv4.2 + KChIP2 $\Delta\text{N2-67}$	rKv4.2 + KChIP3	rKv4.3	rKv4.3 + KChIP1
Peak Current	0.60*	4.5*	6.0*	3.3*	5.8*	3.5*	7.7 $\mu\text{A}$	18.1 $\mu\text{A}$ *
(nA/cell at 50 mV)	$\pm 0.096$	$\pm 0.055$	$\pm 1.1$	$\pm 0.45$	$\pm 1.1$	$\pm 0.99$	$\pm 2.6$	$\pm 3.8$
Peak Current Density	25.5	306.9*	407.2*	196.6*	202.6*	161.7*	---	---
(pA/pF at 50 mV)	$\pm 3.2$	$\pm 57.9$	$\pm 104.8$	$\pm 26.6$	$\pm 27.5$	$\pm 21.8$		
Inactivation time constant	28.2	104.1	129.2	95.1*	109.5*	67.2*	56.3	135.0
(ms, at 50 mV)	$\pm 2.6$	$\pm 10.4$	$\pm 14.2$	$\pm 8.3$	$\pm 9.6$	$\pm 14.1$	$\pm 6.6$	$\pm 15.1$
Recovery from Inactivation Time constant	272.2	53.6*	98.1*	49.5*	36.1*	126.1*	327.0	34.5*

\* Significantly different from control.

**TABLE 2**

Functional effects of KChIPs on other Kv channels

Current Parameter	<i>Xenopus</i> oocytes		<i>Xenopus</i> oocytes	
	HKv1.4	hKv1.4 + 1v	HKv2.1	HKv2.1 + 1v
Peak Current	8.3	6.5	3.7	2.9
( $\mu$ A/cell at 50 MV)	$\pm 2.0$	$\pm 0.64$	$\pm 0.48$	$\pm 0.37$
Inactivation time constant	53.2	58.2	1.9 s	1.7 s
(ms, at 50 mV)	$\pm 2.8$	$\pm 6.6$	$\pm 0.079$	0.078
Recovery from Inactivation time constant (sec, at -80 mV)	1.9	1.6	7.6	7.7
Activation $V_{1/2}$ (mV)	-21.0	-20.9	12.0	12.4
Steady-state Inactivation $V_{1/2}$ (mV)	-48.1	-47.5	-25.3	-23.9

5

**EXAMPLE 11: EFFECTS OF KChIP1 ON SURFACE EXPRESSION OF  
KV4- $\alpha$  SUBUNITS IN COS-1 CELLS**

To examine the ability of KChIP1 to enhance the surface expression of Kv4 channels, the ability of KChIP1 to promote the formation of surface co-clusters of Kv4  
10 channels and PSD-95 was monitored. PSD-95 is used to facilitate the visualization of the complex.

To facilitate the interaction between Kv4.3 and PSD-95, a chimeric Kv4.3 subunit (Kv4.3ch) was generated in which the C-terminal 10 amino acids from rKv1.4 (SNAKAVETDV, SEQ ID NO:73) were appended to the C-terminus of Kv4.3. The C-terminal 10 amino acids from rKv1.4 were used because they associate with PSD-95 and confer the ability to associate with PSD-95 to the Kv4.3 protein when fused to the Kv4.3 C-terminus. Expression of Kv4.3ch in COS-1 cells revealed that the Kv4.3ch polypeptide was trapped in the perinuclear cytoplasm, with minimal detectable Kv4.3ch immunoreactivity at the outer margins of the cell. When Kv4.3ch was co-expressed with PSD-95, PSD-95 became trapped in the perinuclear cytoplasm and co-localized with Kv4.3ch. However, when KChIP1 was co-expressed with Kv4.3ch and PSD-95, large plaque-like surface co-clusters of Kv4.3ch, KChIP1 and PSD-95 were observed. Triple-label immunofluorescence confirmed that these surface clusters contain all three polypeptides, and reciprocal co-immunoprecipitation analyses indicated that the three polypeptides are co-associated in these surface clusters. Control experiments indicated that KChIP1 does not interact with PSD-95 alone, and does not co-localize with Kv1.4 and PSD-95 in surface clusters. Taken together, these data indicate that KChIP1 may promote the transit of the Kv4.3 subunits to the cell surface.

#### EXAMPLE 12: CHARACTERIZATION OF THE PCIP PROTEINS

In this example, the amino acid sequences of the PCIP proteins were compared to amino acid sequences of known proteins and various motifs were identified.

The 1v polypeptide, the amino acid sequence of which is shown in SEQ ID NO:3 is a novel polypeptide which includes 216 amino acid residues. Domains that are putatively involved in calcium binding (Linse, S. and Forsen, S. (1995) *Advances in Second Messenger and Phosphoprotein Research* 30, Chapter 3, p89-151, edited by Means, AR., Raven Press, Ltd., New York), were identified by sequence alignment (see Figure 21).

The 8t polypeptide, the amino acid sequence of which is shown in SEQ ID NO:30 is a novel polypeptide which includes 225 amino acid residues. Calcium binding domains that are putatively involved in calcium binding (Linse, S. and Forsen, S. (1995) *Advances in Second Messenger and Phosphoprotein Research* 30, Chapter 3,

p89-151, edited by Means, AR., Raven Press, Ltd., New York), were identified by sequence alignment (see Figure 21).

The 9q polypeptide is a novel polypeptide which includes calcium binding domains that are putatively involved in calcium binding (Linse, S. and Forsen, S. (1995) *Advances in Second Messenger and Phosphoprotein Research* 30, Chapter 3, p89-151, edited by Means, AR., Raven Press, Ltd., New York (see Figure 21).

The p19 polypeptide is a novel polypeptide which includes calcium binding domains that are putatively involved in calcium binding (Linse, S. and Forsen, S. (1995) *Advances in Second Messenger and Phosphoprotein Research* 30, Chapter 3, p89-151, edited by Means, AR., Raven Press, Ltd., New York (see Figure 21).

A BLASTN 2.0.7 search (Altschul *et al.* (1990) *J. Mol. Biol.* 215:403) of the nucleotide sequence of rat 1vl revealed that the rat 1vl is similar to the rat cDNA clone RMUAH89 (Accession Number AA849706). The rat 1 vl nucleic acid molecule is 98% identical to the rat cDNA clone RMUAH89 (Accession Number AA849706) over nucleotides 1063 to 1488.

A BLASTN 2.0.7 search (Altschul *et al.* (1990) *J. Mol. Biol.* 215:403) of the nucleotide sequence of human 9ql revealed that the human 9ql is similar to the human cDNA clone 1309405 (Accession Number AA757119). The human 9 ql nucleic acid molecule is 98% identical to the human cDNA clone 1309405 (Accession Number AA757119) over nucleotides 937 to 1405.

A BLASTN 2.0.7 search (Altschul *et al.* (1990) *J. Mol. Biol.* 215:403) of the nucleotide sequence of mouse P19 revealed that the mouse P19 is similar to the *Mus musculus* cDNA clone MNCb-7005 (Accession Number AU035979). The mouse P19 nucleic acid molecule is 98% identical to the *Mus musculus* cDNA clone MNCb-7005 (Accession Number AU035979) over nucleotides 1 to 583.

### **EXAMPLE 13:      EXPRESSION OF RECOMBINANT PCIP PROTEINS IN BACTERIAL CELLS**

In this example, PCIP is expressed as a recombinant glutathione-S-transferase (GST) fusion polypeptide in *E. coli* and the fusion polypeptide is isolated and characterized. Specifically, PCIP is fused to GST and this fusion polypeptide is expressed in *E. coli*, *e.g.*, strain BI21. Expression of the GST-PCIP fusion protein in

BI21 is induced with IPTG. The recombinant fusion polypeptide is purified from crude bacterial lysates of the induced BI21 strain by affinity chromatography on glutathione beads. Using polyacrylamide gel electrophoretic analysis of the polypeptide purified from the bacterial lysates, the molecular weight of the resultant fusion polypeptide is  
5 determined.

Rat 1v and 9ql were cloned into pGEX-6p-2 (Pharmacia). The resulting recombinant fusion proteins were expressed in *E. coli* cells and purified following art known methods (described in, for example, *Current Protocols in Molecular Biology*, eds. Ausubel *et al.* John Wiley & Sons: 1992). The identities of the purified proteins  
10 were verified by western blot analysis using antibodies raised against peptide epitopes of rat 1v and 9ql.

#### EXAMPLE 14:      **EXPRESSION OF RECOMBINANT PCIP PROTEINS IN COS CELLS**

15      To express the PCIP gene in COS cells, the pcDNA/Amp vector by Invitrogen Corporation (San Diego, CA) is used. This vector contains an SV40 origin of replication, an ampicillin resistance gene, an *E. coli* replication origin, a CMV promoter followed by a polylinker region, and an SV40 intron and polyadenylation site. A DNA fragment encoding the entire PCIP protein and an HA tag (Wilson *et al.* (1984) *Cell*  
20 37:767) or a FLAG tag fused in-frame to its 3' end of the fragment is cloned into the polylinker region of the vector, thereby placing the expression of the recombinant protein under the control of the CMV promoter.

To construct the plasmid, the PCIP DNA sequence is amplified by PCR using two primers. The 5' primer contains the restriction site of interest followed by  
25 approximately twenty nucleotides of the PCIP coding sequence starting from the initiation codon; the 3' end sequence contains complementary sequences to the other restriction site of interest, a translation stop codon, the HA tag or FLAG tag and the last 20 nucleotides of the PCIP coding sequence. The PCR amplified fragment and the pCDNA/Amp vector are digested with the appropriate restriction enzymes and the  
30 vector is dephosphorylated using the CIAP enzyme (New England Biolabs, Beverly, MA). Preferably the two restriction sites chosen are different so that the PCIP gene is inserted in the correct orientation. The ligation mixture is transformed into *E. coli* cells

(strains HB101, DH5a, SURE, available from Stratagene Cloning Systems, La Jolla, CA, can be used), the transformed culture is plated on ampicillin media plates, and resistant colonies are selected. Plasmid DNA is isolated from transformants and examined by restriction analysis for the presence of the correct fragment.

5 COS cells are subsequently transfected with the PCIP-pcDNA/Amp plasmid DNA using the calcium phosphate or calcium chloride co-precipitation methods, DEAE-dextran-mediated transfection, lipofection, or electroporation. Other suitable methods for transfecting host cells can be found in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989. The expression of the PCIP polypeptide is detected by radiolabelling ( $^{35}\text{S}$ -methionine or  $^{35}\text{S}$ -cysteine available from NEN, Boston, MA, can be used) and immunoprecipitation (Harlow, E. and Lane, D. *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988) using an HA specific monoclonal antibody. Briefly, the cells are labelled for 8 hours with  $^{35}\text{S}$ -methionine (or  $^{35}\text{S}$ -cysteine). The culture media are then collected and the cells are lysed using detergents (RIPA buffer, 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% DOC, 50 mM Tris, pH 7.5). Both the cell lysate and the culture media are precipitated with an HA specific monoclonal antibody. Precipitated polypeptides are then analyzed by SDS-PAGE.

20 Alternatively, DNA containing the PCIP coding sequence is cloned directly into the polylinker of the pCDNA/Amp vector using the appropriate restriction sites. The resulting plasmid is transfected into COS cells in the manner described above, and the expression of the PCIP polypeptide is detected by radiolabelling and immunoprecipitation using a PCIP specific monoclonal antibody.

25 Rat 1v was cloned into the mammalian expression vector pRBG4. Transfections into COS cells were performed using LipofectAmine Plus (Gibco BRL) following the manufacturer's instructions. The expressed 1v protein was detected by immunocytochemistry and/or western blot analysis using antibodies raised against 1v in rabbits or mice.

30

**EXAMPLE 15: IDENTIFICATION AND CHARACTERIZATION OF  
HUMAN FULL LENGTH P19**

The human full length p19 sequence was identified using RACE PCR. The sequence of p19 (also referred to as KChIP3) is shown in Figure 16. The amino acid  
5 sequence of human p19 is 92% identical to the mouse p19 gene (SEQ ID NO:35).

TBLASTN searches using the protein sequence of human p19 revealed that human p19 is homologous to two sequences, Calsenilin (described in (1998) *Nature Medicine* 4: 1177-1181) and DREAM, a Ca<sup>2+</sup>-dependent regulator of prodynorphin and c-fos transcription (described in Carrion *et al.* (1999) *Nature* 398: 80-84). Human p19 is  
10 100% identical at the nucleotide level to Calsenilin (but extends 3' to the published sequence) and 99% identical at the nucleotide level to DREAM.

The ability of p19 (as well as other PCIP family members) to co-localize with presenilin and act as transcription factors is determined using art known techniques such as northern blots, *in situ* hybridization,  $\beta$ -gal assays, DNA mobility assays (described in,  
15 for example, Carrion *et al.* (1999) *Nature* 398:80) and DNA mobility supershift assays, using antibodies specific for KChIPs.

Other assays suitable for evaluating the association of PCIP family members with presenilins is co-immunoprecipitation (described in, for example, Buxbaum *et al.* (1998) *Nature Medicine* 4:1177).

20

**EXAMPLE 16: IDENTIFICATION AND CHARACTERIZATION OF  
MONKEY KChIP4**

In this example, the identification and characterization of the genes encoding monkey KChIP4a (jlkbd352e01t1) and alternatively spliced monkey KChIP4b  
25 (jlkbb231c04t1), KChIP4c (jlksa053c02), and KChIP4d (jlkx015b10) is described. TBLASTN searches in proprietary databases with the sequence of the known PCIP family members, lead to the identification of four clones jlkbb231c04t1, jlkbd352e01t1, jlksa053c02, and jlkx015b10. The four monkey clones were obtained and sequenced.

The sequences of proprietary monkey clones jlkbb231c04t1 and jlkbd352e01t1  
30 were found to correspond to alternately spliced variants of an additional PCIP family member, referred to herein as KChIP4. Clone jlkbb231c04t1 contains a 822bp deletion relative to jlkbd352e01t1 (presumably due to splicing out of an exon), resulting in the

loss of the final EF hand domain. In clone jlkbd352e01t1, the final EF hand domain is preserved, and the C-terminus is highly homologous to that of PCIP family members 1v, 9ql, and p19. Overall identity in the homologous C-termini among KChIP4, 1v, 9ql, and p19 ranged from 71%-80% at the amino acid level (alignments were performed using the CLUSTALW).

Monkey KChIP4c and KChIP4d were discovered by BLASTN search using monkey KChIP4a as a query for searching a proprietary database.

The nucleotide sequence of the monkey KChIP4a cDNA and the predicted amino acid sequence of the KChIP4a polypeptide are shown in Figure 23 and in SEQ ID NOs:48 and 49, respectively.

The nucleotide sequence of the monkey KChIP4b cDNA and the predicted amino acid sequence of the KChIP4b polypeptide are shown in Figure 24 and in SEQ ID NOs:50 and 51, respectively.

The nucleotide sequence of the monkey KChIP4c cDNA and the predicted amino acid sequence of the KChIP4c polypeptide are shown in Figure 35 and in SEQ ID NOs:69 and 70, respectively.

The nucleotide sequence of the monkey KChIP4d cDNA and the predicted amino acid sequence of the KChIP4d polypeptide are shown in Figure 36 and in SEQ ID NOs:71 and 72, respectively.

Figure 37 depicts an alignment of the protein sequences of KChIP4a, KChIP4b, KChIP4c, and KChIP4d.

Rat KChIP4 is predominantly expressed in the brain, and weakly in the kidney, but not in the heart, brain, spleen, lung, liver, skeletal muscle or testes, as indicated by northern blot experiments in which a northern blot purchased from Clontech was probed with a DNA fragment from the 3'-untranslated region of rat KChIP4.

#### **EXAMPLE 17: IDENTIFICATION AND CHARACTERIZATION OF HUMAN AND RAT 33b07**

In this example, the identification and characterization of the genes encoding rat and human 33b07 is described. Partial rat 33b07 (clone name 9o) was isolated as a positive clone from the yeast two-hybrid screen described above, using rKv4.3N as bait. The full length rat 33b07 clone was identified by mining of proprietary databases.



The nucleotide sequence of the full length rat 33b07 cDNA and the predicted amino acid sequence of the rat 33b07 polypeptide are shown in Figure 26 and in SEQ ID NOs:52 and 53, respectively. The rat 33b07 cDNA encodes a protein having a molecular weight of approximately 44.7 kD and which is 407 amino acid residues in length.

Rat 33b07 binds rKv4.3N and rKv4.2N with slight preference for rKv4.2N in yeast 2-hybrid assays. In contrast, rat 33b07 does not bind rKv1.1N, indicating that the rat 33b07-Kv4N interaction is specific.

Rat 33b07 is expressed predominantly in the brain as determined by northern blot analysis.

The human 33b07 ortholog (clone 106d5) was also identified by mining of proprietary databases. The nucleotide sequence of the full length human 33b07 cDNA and the predicted amino acid sequence of the human 33b07 polypeptide are shown in Figure 27 and in SEQ ID NOs:54 and 55, respectively. The human 33b07 cDNA encodes a protein having a molecular weight of approximately 45.1 kD and which is 414 amino acid residues in length.

Human 33b07 is 99% identical to the human KIAA0721 protein (GenBank Accession Number: AB018264) at the amino acid level. However, GenBank Accession Number: AB018264 does not have a functional annotation. Human 33b07 is also homologous to Testes-specific (Y-encoded) proteins (TSP(Y)s), SET, and Nucleosome Assembly Proteins (NAPs). The human 33b07 is 38% identical to human SET protein (GenBank Accession Number Q01105=U51924) over amino acids 204 to 337 and 46% identical over amino acids 334 to 387.

Human SET is also called HLA-DR associated protein II (PHAPII) (Hoppe-Seyler (1994) *Biol. Chem.* 375:113-126) and in some cases is associated with acute undifferentiated leukemia (AUL) as a result of a translocation event resulting in the formation of a SET-CAN fusion gene (Von Lindern M. *et al.* (1992) *Mol. Cell. Biol.* 12:3346-3355). An alternative spliced form of SET is also called Template Activating Factor-I alpha (TAF). TAF is found to be associated with myeloid leukemogenesis (Nagata K. *et al.* (1995) *Proc. Natl. Acad. Sci. U.S.A.* 92 (10), 4279-4283). Human SET is also a potent protein inhibitor of phosphatase 2A (Adachi Y. *et al.* (1994) *J. Biol. Chem.* 269:2258-2262). NAPs may be involved in modulating chromatin formation and

contribute to regulation of cell proliferation (Simon H.U. *et al.* (1994) *Biochem. J.* 297, 389-397).

Thus, due to its homology to the above identified proteins, 33b07 may function as a protein inhibitor of phosphatase, an oncogene, and/or a chromatin modulator. The homology of 33b07 to SET, a protein phosphatase inhibitor, is of particular interest. Many channels, in particular the Kv4 channels (with which 33b07 is associated), are known to be regulated by phosphorylation by PKC and PKA ((1998) *J. Neuroscience* 18(10): 3521-3528; *Am J Physiol* 273: H1775-86 (1997)). Thus, 33b07 may modulate Kv4 activity by regulating the phosphorylation status of the potassium channel.

#### **EXAMPLE 18: IDENTIFICATION AND CHARACTERIZATION OF RAT 1p**

In this example, the identification and characterization of the gene encoding rat 1p is described. Partial rat 1p was isolated as a positive clone from the yeast two-hybrid screen described above, using rKv4.3N as a bait.

The nucleotide sequence of the partial length rat 1p cDNA and the predicted amino acid sequence of the rat 1p polypeptide are shown in Figure 28 and in SEQ ID NOs:56 and 57, respectively. The rat 1p cDNA encodes a protein having a molecular weight of approximately 28.6 kD and which is 267 amino acid residues in length.

Rat 1p binds rKv4.3N and rKv4.2N with slight preference for rKv4.3N in yeast two-hybrid assays. In contrast, 1p does not bind rKv1.1N, indicating that the 1p-Kv4N interaction is specific.

Rat 1p is predominantly expressed in the brain as determined by northern blot analysis.

A BLASTP 1.4 search, using a score of 100 and a word length of 3 (Altschul *et al.* (1990) *J. Mol. Biol.* 215:403) of the amino acid sequences of rat 1p revealed that rat 1p is similar to the human Restin (GenBank Accession Number P30622; also named cytoplasmic linker protein-170 alpha-2 (CLIP-170), M97501)). The rat 1p protein is 58% identical to the human Restin over amino acid residues 105 to 182, 55% identical to the human Restin over amino acid residues 115 to 186, 22% identical to the human Restin over amino acid residues 173 to 246, 22% identical to the human Restin over

amino acid residues 169 to 218, and 58% identical to the human Restin over amino acid residues 217 to 228.

Restin is also named Reed-Sternberg intermediate filament associated protein. Reed-Sternberg cells are the tumoral cells diagnostic for Hodgkin's disease. It is suggested that Restin overexpression may be a contributing factor in the progression of Hodgkin's disease (Bilbe G. *et al.* (1992) *EMBO J.* 11: 2103-13) and Restin appears to be an intermediate filament associated protein that links endocytic vesicles to microtubules (Pierre P, *et al.* (1992) *Cell* 70 (6), 887-900).

The cytoskeleton regulates the activity of potassium channels (see, for example, Honore E, *et al.* (1992) *EMBO J.* 11:2465-2471 and Levin G, *et al.* (1996) *J. Biol. Chem.* 271:29321-29328), as well as the activity of other channels, *e.g.*,  $\text{Ca}^{++}$  channels (Johnson B.D. *et al.* (1993) *Neuron* 10:797-804); or  $\text{Na}^{+}$  channels (Fukuda J. *et al.* (1981) *Nature* 294:82-85).

Accordingly, based on its homology to the Restin protein, the rat 1p protein may be associated with the cytoskeleton and may modulate the activity of potassium channels, *e.g.*, Kv4, via its association to the cytoskeleton.

#### EXAMPLE 19: IDENTIFICATION AND CHARACTERIZATION OF RAT 7s

In this example, the identification and characterization of the gene encoding rat 7s is described. Partial rat 7s was isolated as a positive clone from the yeast two-hybrid screen described above, using rKv4.3N as a bait. Rat 7s is the rat ortholog of the human vacuolar H(+)-ATPase catalytic subunit A (Accession Number P38606 and B46091) described in, for example, van Hille B. *et al.* (1993) *J. Biol. Chem.* 268 (10), 7075-7080.

The nucleotide sequence of the partial length rat 7s cDNA and the predicted amino acid sequence of the rat 7s polypeptide are shown in Figure 29 and in SEQ ID NOs:58 and 59, respectively. The rat 7s cDNA encodes a protein having a molecular weight of approximately 28.6 kD and which is 270 amino acid residues in length.

Rat 7s binds rKv4.3N and rKv4.2N with preference for rKv4.3N in yeast two-hybrid assays. In contrast, 7s does not bind rKv1.1N, indicating that the 7s-Kv4N interaction is specific.

Rat 7s is expressed at significantly higher levels in the brain and the kidney than in the lung, liver, heart, testes, and skeletal muscle, as determined by northern blot analysis.

**5    EXAMPLE 20:            IDENTIFICATION AND CHARACTERIZATION OF  
                                     RAT 29x AND 25r**

In this example, the identification and characterization of the gene encoding rat 29x is described. Rat 29x was isolated as a positive clone from the yeast two-hybrid screen described above, using rKv4.3N as a bait. Rat 25r is a splice variant of 29x.

10   They differ in the 5' untranslated region, but are identical in the coding region and at the amino acid level.

The nucleotide sequence of the rat 29x cDNA and the predicted amino acid sequence of the rat 29x polypeptide are shown in Figure 30 and in SEQ ID NOs:60 and 61, respectively. The rat 29x cDNA encodes a protein having a molecular weight of  
15   approximately 40.4 kD and which is 351 amino acid residues in length.

The nucleotide sequence of the rat 25r cDNA is shown in Figure 31 and in SEQ ID NO:62. The rat 25r cDNA encodes a protein having a molecular weight of approximately 40.4 kD and which is 351 amino acid residues in length.

Rat 29x is expressed in the spleen, lung, kidney, heart, brain, testes, skeletal  
20   muscle and liver, with the highest level of expression being in the spleen and the lowest being in the liver.

Rat 29x binds rKv4.3N and rKv4.2N with slight preference for rKv4.3N in yeast two-hybrid assays. In contrast, 29x does not bind rKv1.1N, indicating that the 29x-Kv4N interaction is specific.

25   Rat 29x is identical at the amino acid level to rat SOCS-1 (Suppressor Of Cytokine Signaling) described in Starr R. *et al.* (1997) *Nature* 387: 917-921; to JAB described in Endo T.A. *et al.* (1997) *Nature* 387: 921-924; and to SSI-1 (STAT-induced STAT inhibitor-1) described in Naka T. *et al.* (1997) *Nature* 387:924-928. These proteins are characterized in that they have an SH2 domain, bind to and inhibit JAK  
30   kinase, and, as a result, regulate cytokine signaling.

As used herein, the term "SH2 domain", also referred to a Src Homology 2 domain, includes a protein domain of about 100 amino acids in length which is involved in binding of phosphotyrosine residues, *e.g.*, phosphotyrosine residues in other proteins. The target site is called an SH2-binding site. The SH2 domain has a conserved 3D structure consisting of two alpha helices and six to seven beta-strands. The core of the SH2 domain is formed by a continuous beta-meander composed of two connected beta-sheets (Kuriyan J. *et al.* (1997) *Curr. Opin. Struct. Biol.* 3:828-837). SH2 domains function as regulatory modules of intracellular signaling cascades by interacting with high affinity to phosphotyrosine-containing target peptides in a sequence-specific and strictly phosphorylation-dependent manner (Pawson T. (1995) *Nature* 373:573-580). Some proteins contain multiple SH2 domains, which increases their affinity for binding to phosphoproteins or confers the ability to bind to different phosphoproteins. Rat 29x contains an SH2 domain at amino acid residues 219-308 of SEQ ID NO:61.

Tyrosine phosphorylation regulates potassium channel activity (Prevarskaya N.B. *et al.* (1995) *J. Biol. Chem.* 270:24292-24299). JAK kinase phosphorylates proteins at tyrosines and is implicated in the regulation of channel activity (Prevarskaya N.B. *et al. supra*). Accordingly, based on its homology to SOCS-1, JAB, and SSI-1, rat 29x may modulate the activity of potassium channels, *e.g.*, Kv4, by modulating JAK kinase activity.

#### **EXAMPLE 21: IDENTIFICATION AND CHARACTERIZATION OF RAT 5p**

In this example, the identification and characterization of the gene encoding rat 5p is described. Rat 5p was isolated as a positive clone from the yeast two-hybrid screen described above, using rKv4.3N as a bait.

The nucleotide sequence of the rat 5p cDNA and the predicted amino acid sequence of the rat 5p polypeptide are shown in Figure 32 and in SEQ ID NOs:63 and 64, respectively. The rat 5p cDNA encodes a protein having a molecular weight of approximately 11.1 kD and which is 95 amino acid residues in length.

Rat 5p binds rKv4.3N and rKv4.2N with similar strength in yeast two-hybrid assays. In contrast, 5p does not bind rKv1.1N, indicating that the 5p-Kv4N interaction is specific.

Rat 5p is expressed in the spleen, lung, skeletal muscle, heart, kidney, brain, liver, and testes, as determined by northern blot analysis.

The rat 5p is identical to rat Calpactin I light chain or P10 (Accession Number P05943). P10 binds and induces the dimerization of annexin II (p36). P10 may function  
5 as a regulator of protein phosphorylation in that the p36 monomer is the preferred target of a tyrosine-specific kinase (Masiakowski P. *et al.* (1998) *Proc. Natl. Acad. Sci. U.S.A.* 85 (4): 1277-1281).

Tyrosine phosphorylation regulates the activity of potassium channels (Prevarskaya N.B. *et al. supra*). Thus, due to its identity to P10, rat 5p may modulate  
10 the activity of potassium channels, *e.g.*, Kv4, by modulating the activity of a tyrosine-specific kinase.

#### **EXAMPLE 22: IDENTIFICATION AND CHARACTERIZATION OF RAT 7q**

15 In this example, the identification and characterization of the gene encoding rat 7q is described. Rat 7q was isolated as a positive clone from the yeast two-hybrid screen described above, using rKv4.3N as a bait. Full length rat 7q was obtained by RACE PCR.

The nucleotide sequence of the rat 7q cDNA and the predicted amino acid  
20 sequence of the rat 7q polypeptide are shown in Figure 33 and in SEQ ID NOs:65 and 66, respectively. The rat 7q cDNA encodes a protein having a molecular weight of approximately 23.5 kD and which is 212 amino acid residues in length.

Rat 7q binds rKv4.3N and rKv4.2N with same strength in yeast two-hybrid assays. In contrast, 7q does not bind rKv1.1N, indicating that the 7q-Kv4N interaction is  
25 specific.

Rat 7q is expressed in the heart, brain, spleen, lung, liver, skeletal muscle, kidney, and testes, as determined by northern blot analysis.

Rat 7q is identical to RAB2 (rat RAS-related protein, Accession Number P05712) at the amino acid level. RAB2 appears to be involved in vesicular traffic and  
30 protein transport (Touchot N. *et al.* (1987) *Proc. Natl. Acad. Sci. U.S.A.* 84 (23): 8210-8214). Accordingly, based on its homology to RAB2, rat 7q may be involved in potassium channel, *e.g.*, Kv4, trafficking.

### EXAMPLE 23: IDENTIFICATION AND CHARACTERIZATION OF RAT 19r

In this example, the identification and characterization of the gene encoding rat  
5 19r is described. Partial rat 19r was isolated as a positive clone from the yeast two-  
hybrid screen described above, using rKv4.3N as a bait. Full length rat 19r was obtained  
by RACE PCR.

The nucleotide sequence of the rat 19r cDNA and the predicted amino acid  
sequence of the rat 19r polypeptide are shown in Figure 34 and in SEQ ID NOs:67 and  
10 68, respectively. The rat 19r cDNA encodes a protein having a molecular weight of  
approximately 31.9 kD and which is 271 amino acid residues in length.

Rat 19r is expressed in the heart, brain, spleen, lung, liver, skeletal muscle,  
kidney, and testes, as determined by northern blot analysis.

Rat 19r binds rKv4.3N and rKv4.2N with slight preference for rKv4.3N in yeast  
15 two-hybrid assays. In contrast, 19r does not bind rKv1.1N, indicating that the 19r-Kv4N  
interaction is specific.

Rat 19r is identical to Rat phosphatidylinositol (PTDINS) transfer protein alpha  
(PTDINSTP, Accession Number M25758 or P16446) described in Dickeson S.K. *et al.*  
(1989) *J. Biol. Chem.* 264:16557-16564. PTDINSTP is believed to be involved in  
20 phospholipase C-beta (PLC-beta) signaling, phosphatidylinositol transfer protein  
(PtdIns-TP) synthesis, secretory vesicle formation, and enhancement of  
phosphatidylinositol 3-kinase (PtdIns 3-kinase) activity (Cunningham E. *et al.* (1995)  
*Curr. Biol.* 5 (7): 775-783; (1995) *Nature* 377 (6549): 544-547; and Panaretou C. *et al.*  
(1997) *J. Biol. Chem.* 272 (4): 2477-2485).

25 Accordingly, based on its homology with PTDINSTP, rat 19r may modulate  
potassium channel, *e.g.*, Kv4, activity via the PLC-beta signaling pathway and/or the  
PtdIns 3-kinase signaling pathway. Rat p19r may also be involved in potassium  
channel, *e.g.*, Kv4, trafficking.

**EXAMPLE 24: CHROMOSOMAL LOCALIZATION OF HUMAN 9q**

In this example, the human PCIP 9q was chromosomally mapped using a radiation hybrid panel (Panel GB4). h9q mapped to a region of chromosome 10q that had been previously shown to contain a linkage with partial epilepsy, namely D10S192: 10q22-q24 (Ottman *et al.* (1995) *Nature Genetics* 10:56-60) (see Figure 43). Based on this observation, the present invention clearly demonstrates that the 9q family of proteins can serve as targets for developing anti-epilepsy drugs and as targets for medical intervention of epilepsy.

Furthermore, h9q mapped to a region of chromosome 10q that had been previously shown to contain a linkage with IOSCA, namely D10S192 and D10S1265: 10q24- Nikali (Genomics 39:185-191 (1997)) (see Figures 42 and 43). Based on this observation, the present invention clearly demonstrates that the 9q family of proteins can serve as targets for developing anti-spinocerebellar ataxia drugs and as targets for medical intervention of spinocerebellar ataxia.

**EXAMPLE 25: ARACHIDONIC ACID MODULATION OF KV4/KChIP CHANNELS**Kinetic modulation of Kv4 current by AA is KChIP-dependent

Arachidonic Acid (AA) was shown to inhibit recombinant Kv4 current expressed in *Xenopus* oocytes (Villarroel, A. and Schwarz, T. L. (1996) *J. Neuroscience* 16:2522-32). However, the modulation was only observed with peak current amplitude whereas the current kinetic parameters were not affected by the presence of AA. In contrast, recordings of membrane patches from hippocampal neurons showed that in addition to suppression of peak amplitude, AA changed the kinetic parameters of the A-current by Kv4 channels (Keros, S. and McBain, C. J. (1997) *J. Neuroscience* 17: 3476-87). Notably, the inactivation time constant was considerably reduced (note: inactivation time constant is inversely correlated to rate of inactivation. Therefore, inactivation was sped up (Keros (1997) *supra*).

In this Example, the hypothesis that KChIPs were the missing auxiliary subunits that accounted for the above kinetic discrepancy was investigated by expressing Kv4 alone or together with KChIPs in both CHO cells and *Xenopus* oocytes, and measuring their inactivation time constants (using art known techniques as described in, for example, An *et al.* (2000) *Nature* 403:553-6; Keros, S. and McBain, C. J. (1997) *J. Neuroscience* 17: 3476-87; and Villarroel, A. and Schwarz, T. L. (1996) *J. Neuroscience* 16:2522-32).



The kinetic modulation of Kv4 by AA was demonstrated to be KChIP-dependent (Table 3). When Kv4.2 was expressed alone in CHO cells, the inactivation time constant of the resulting current was unchanged in the absence or presence of 10  $\mu$ M of AA ( $32 \pm 3$  vs.  $32 \pm 2$  milliseconds(ms)  $\pm$  standard error mean (SEM)). In contrast, when co-expressed with KChIP1, the inactivation time constant of Kv4.2 current was decreased from  $88 \pm 8$  ms in the absence of AA to  $37 \pm 3$  ms in the presence of 10  $\mu$ M of AA. Similar results were obtained with KChIP1 (Table 4) and KChIP2 in *Xenopus* oocytes. These results demonstrate that kinetic modulation of Kv4 current by AA is dependent on the presence of KChIPs. The kinetic change of Kv4/KChIPs in the presence of AA is consistent with that described on neuronal membranes [Keros (1997) *supra*] supporting the notion that KChIPs are the endogenous subunits of Kv4-underlying current.

It is noted that AA also suppressed peak amplitude of Kv4/KChIP current in both CHO cells and *Xenopus* oocytes (Tables 3 and 4). This indicates that modulation of peak amplitude of Kv4 currents is independent of KChIPs.

TABLE 3. AA modulation of Kv4 and Kv4/KChIP1 currents in CHO cells.

	Kv4.2	Kv4.2	KV4.2/KChIP 1	KV4.2/KChIP 1
	0 $\mu$ M AA	10 $\mu$ M AA	0 $\mu$ M AA	10 $\mu$ M AA
Inactivation time constant (ms $\pm$ SEM)	$32 \pm 3$	$32 \pm 2$	$88 \pm 8$	$37 \pm 3$
Peak amplitude (pA $\pm$ SEM)	$620 \pm 80$	$336 \pm 82$	$4539 \pm 448$	$2827 \pm 496$

The arachidonic acid effects on the A-current were also investigated in a neuronal system (cultured primary cerebellar granule neurons) where both Kv4 and KChIPs are present. TEA (10 mM) was applied to block a small sustained outward component. Inactivation time constants of the A current in the absence and presence of 10  $\mu$ M arachidonic acid were  $44 \pm 5$  ms and  $21 \pm 3$  ms (mean  $\pm$  SEM), respectively. The corresponding peak amplitude was reduced from  $2.0 \pm 0.6$  nA to  $1.2 \pm 0.4$  nA. These results confirm that arachidonic acid modulates both Kv4 A-current current amplitude and kinetics in native cells.

Arachidonic acid modulation of Kv4/KChIP current is concentration-dependent and reversible

The effects of different concentrations of arachidonic acid on Kv4/KChIP current was studied in *Xenopus* oocytes. Because the physiological concentrations of arachidonic acid are often under 10  $\mu$ M (Needleman, *et al.*, 1986 Annu Rev Biochem 55:69-102; Anderson and Welsh, 1990, Proc Natl Acad Sci U S A 87:7334-8; Meves, 1994, Prog Neurobiol 43:175-86), arachidonic acid was tested in the 1-10  $\mu$ M range. The concentration-dependent block of peak amplitude of the Kv4.3 current was independent of the presence of KChIP1 (see Figure 64A). Further, the slope of amplitude reduction as a function of increasing concentrations was very similar with or without the presence of KChIPs. Peak current block did not appear to saturate up to 10  $\mu$ M. Villarroel and Schwarz, (1996) *J. Neurosci* 16:2522-32 reported that the IC<sub>50</sub> of arachidonic acid on Kv4  $\alpha$  subunits was approximately 8  $\mu$ M in oocytes. The inactivation time constant in the absence of KChIP1 was unchanged at all arachidonic acid concentrations tested. However, in the presence of KChIP1, inactivation time constant decreased in a concentration-dependent manner (see Figure 64B).

The onset of the KChIP-dependent acceleration of inactivation and the KChIP-independent current block of Kv4.3 by 10  $\mu$ M arachidonic acid was almost immediate (Figure 65). At least part of the slight delay (14 seconds) was attributed to the transit of solution from the reservoir to the recording chamber. The amplitude block developed gradually over time (Figure 65A). The presence of KChIP1 did not substantially alter either the percent decrease or the rate of current block over time, nor did it change the rate of recovery of Kv4.3 current amplitude over time (Figure 65A). In contrast to the gradual development of amplitude block, the KChIP1-dependent effect on Kv4 kinetics appeared much more rapidly following arachidonic acid perfusion, and tended to plateau quickly (Figure 65B). When arachidonic acid was washed out, Kv4.3 current amplitude and inactivation time constants fully recovered with similar rates in the presence of KChIP1 (compare Figures 65A and 65B). The two small inflections in the Kv4.3 alone plot in panel B were artifacts due to buffer changes.

#### Modulation of Kv4/KChIP current by other fatty acids

Certain fatty acids were shown previously to mimic the effects of arachidonic acid on Kv4 current in *Xenopus* oocytes when Kv4  $\alpha$  was expressed alone (Villarroel and Schwarz, *J Neurosci* 16:2522-32 (1996)). Thus, the fatty acid selectivity for Kv4 current in the presence of KChIPs was investigated. Arachidonic acid is a 20-carbon fatty acid carrying four *cis* double bonds with the first double bond at C5 (20:4 c5). The following arachidonic acid analogs with distinct structural features were studied:  $\gamma$ -linolenic acid (18:3 c9) has three *cis* double bonds instead of four double bonds, linolelaidic acid (18:2 t9) has two *trans* double bonds instead of four *cis* double bonds, 5,8,11,14-eicosatetraynoic acid (ETYA, 20:4 n5) has four triple bonds instead of double

bonds found in arachidonic acid (n indicates position of the first triple bond), and 5,8,11-eicosatriynoic acid (ETI, 20:3 n5) has three triple bonds. Figure 66A shows that the peak amplitude of Kv4.3 current was inhibited significantly compared with no-fatty acid control by 10  $\mu$ M of  $\gamma$ -linolenic acid, ETI, ETYA, and arachidonic acid, independent of the presence of KChIP1. The percent inhibition of amplitude of Kv4 alone and Kv4/KChIP was not significantly different for these fatty acids. A small, statistically significant block of Kv4 current amplitude by 10  $\mu$ M linolelaidic acid was observed in the presence of KChIP1 but not absence of KChIP1 when the values were compared to their respective controls. However, there was no significant difference when comparing Kv4.3 and Kv4.3/KChIP KChIP1.

In the absence of KChIP1 none of the fatty acids tested showed a statistically significant effect on Kv4.3 inactivation time constant (Figure 66B). Only those fatty acids that caused a substantial current block independently of KChIPs ( $\gamma$ -linolenic acid, ETI, ETYA, and arachidonic acid) reduced Kv4.3 inactivation time constant when co-expressed with KChIP1. Linolelaidic acid, which showed only a modest KChIP-dependent Kv4.3 current block, did not affect the Kv4.3 inactivation time constant (Figure 66B). Therefore, certain long chain fatty acids can imitate arachidonic acid to modulate Kv4 current kinetics in a KChIP-dependent manner. In general, there is good connection in the ability for a given fatty acid to block peak amplitude and modify kinetics of the reconstituted Kv4/KChIP current.

#### Arachidonic acid does not disrupt association of Kv4 and KChIP

For this experiment, the following assays were used.

##### *In vitro binding assay*

The N-terminal domain of rat Kv4.3 was expressed as a GST fusion (GST-Kv4.3N) and purified from E.coli essentially following protocols provided by Amersham Pharmacia Biotech (Piscataway, New Jersey). Recombinant rat KChIP1 protein was first expressed and purified as a GST-fusion, then the GST moiety was cleaved using PreScission protease (Amersham Pharmacia Biotech) to give rise to the free KChIP1 protein. Both GST-Kv4.3N and KChIP proteins were >95% pure as estimated by coomassie stain of denaturing gels. In vitro binding assays were performed using a Biacore 3000 from Biacore AB in Uppsala, Sweden. The experiments were performed in phosphate buffered saline (PBS), pH 7.4, with 1 mM  $\text{CaCl}_2$  and 0.05% polysorbate P-20. Anti-GST antibody (Biacore AB) was coupled to 3 flowcells of a CM-5 chip (Biacore AB) at a level of 2000 resonance units (RUs) using amine coupling. The final flowcell was activated and blocked with ethanolamine to use as a reference control surface. The GST-Kv4.3N terminal domain was captured on two of the anti-GST flowcells and GST alone was bound to the third anti-GST flowcell at levels of 150

RUs. Purified KChIP1 at 1  $\mu$ M in the presence and absence of 10  $\mu$ M arachidonic acid was then injected over all four flowcells. Arachidonic acid (10  $\mu$ M) alone was also injected. Data are shown as GST reference-subtracted sensograms.

#### 5 *Yeast 2-hybrid strains and growth assays*

Diploid strains containing bait (the N-terminal domain of Kv4.3 or the empty vector pGBT9) and fish (KChIP1) plasmids were obtained as described in (An, *et al.*, 2000). For synchronization, strains were grown to saturation before they were inoculated at equal OD600 value into 5 ml of synthetic complete-TrpLeuHis drop-out  
10 (SC-WLH) medium that selects for interaction-dependent growth or 5 ml of SC-WL medium that is nonselective in the presence or absence of 10  $\mu$ M ETYA. 5 mM 3-AT (3-amino-1,2,4-triazole) was included in the media to suppress weak self-activating activity from the Kv4.3 N-terminal domain bait. Cultures were grown for 17 hours at 30°C and OD600 values were read by a spectrophotometer.

15

To test the hypothesis that arachidonic acid acts by interfering with the binding between Kv4 and KChIPs, the surface plasmon resonance measurement (Biosensor) was first used to monitor the association and dissociation phases of Kv4-KChIP interaction in the presence and absence of arachidonic acid. The intracellular N-terminal domain of  
20 Kv4.3 was expressed as a GST fusion protein (GST-Kv4.3N) and immobilized to the surface of a Biosensor chip. Recombinant KChIP1 protein was passed over the chip surface in the presence and absence of 10  $\mu$ M arachidonic acid. As shown in Figure 67A, KChIP1 protein was bound to the GST-Kv4.3N surface but a qualitative difference was not observed in either the on- or the off- phase of the association of KChIP1 and the  
25 Kv4.3 N-terminal domain. The Biosensor results were further confirmed in the yeast 2-hybrid system where Kv4-KChIP interaction-dependent growth in the selective SC-WLH medium was not affected by 10  $\mu$ M ETYA (Figure 67B). ETYA instead of arachidonic acid was used in these experiments because, while both ETYA and arachidonic acid affect Kv4 current nearly identically, ETYA is non-metabolizable, and  
30 is, thus, better suited for this experiment. Taken together, the results show that the fatty acids tested do not disrupt association between Kv4 and KChIPs.

#### Kv4/KChIP is more sensitive to AA modulation than is Kv1.1/Kv $\beta$ 1

The pore-forming alpha subunits of ion channels, including those of potassium  
35 channels, often do not work alone. They associate with auxiliary subunits and these auxiliary subunits can change channel activities dramatically. Therefore, it is more useful to study alpha subunits in combination with their auxiliary subunits as the physiologically relevant channels are complexes of alpha-auxiliary subunits.

Expressed alone, the recombinant Kv4 alpha subunits were shown to be by far more sensitive to AA inhibition than alpha subunits of several other voltage-gated potassium channels (e.g., Kv1.1) (Villarroel (1996) *supra*). However, this paper examined AA modulation of only the alpha subunits of the channels. It was not known whether Kv4 current would still be more sensitive to AA modulation than would be other channel currents if all channels were to be tested in the presence of their cognate auxiliary subunits.

In this example, the foregoing was tested by measuring two alpha/auxiliary complexes: Kv4.3/KChIP1 and Kv1.1/Kvβ1. (Kvβ1, which is one of the classic potassium channel beta subunits, dramatically changes Kv1.1 kinetics). Kv4.3/KChIP1 and Kv1.1/Kvβ1 were expressed respectively in *Xenopus* oocytes and their resulting currents were recorded in the presence or absence of 10 μM AA. The results indicated that the peak amplitude of Kv1.1/Kvβ1 current was not significantly increased in the presence of 10 μM of AA ( $11 \pm 4$  to  $14 \pm 1$  μA), whereas the peak amplitude of Kv4.3/KChIP1 was drastically decreased ( $44 \pm 10$  to  $21 \pm 4$  μA, Table 4). Kinetically, Kv4.3/KChIP1 was much more sensitive to AA modulation than was Kv1.1/Kvβ1 (Table 4). While 10 μM AA did not cause a statistically significant decrease of the inactivation time constant of Kv1.1/Kvβ1 ( $11 \pm 1$  to  $9 \pm 1$  ms), the same concentration of AA considerably decreased that of Kv4.3/KChIP1 from  $104 \pm 7$  to  $55 \pm 4$  ms). These results indicate that AA more readily modulates both the kinetics and amplitude of Kv4/KChIPs potassium currents in native neurons than the kinetics and amplitude of Kv1.1/Kvβ1.

**TABLE 4.** AA modulation of Kv4, Kv4/KChIP1, Kv1.1, Kv1.1/Kvβ1 currents in *Xenopus* oocytes.

	Kv4.3	Kv4.3	Kv4.3/KChIP1	Kv4.3/KChIP1	Kv1.1	Kv1.1	Kv1.1/Kvβ1	Kv1.1/Kvβ1
	0 μM AA	10 μM AA	0 μM AA	10 μM AA	0 μM AA	10 μM AA	0 μM AA	10 μM AA
Inactivation time constant (ms ± SEM)	$75 \pm 7$	$66 \pm 6$	$104 \pm 7$	$55 \pm 4$	N.A.	N.A.	$11 \pm 1$	$9 \pm 1$
Peak amplitude (μA ± SEM)	$30 \pm 7$	$13 \pm 1$	$44 \pm 10$	$21 \pm 4$	$19 \pm 2$	$21 \pm 3$	$11 \pm 4$	$14 \pm 1$

#### EXAMPLE 26: K-CHANNEL INTERACTING PROTEIN-2 (KChIP2) SPLICE VARIANTS, CHROMOSOMAL ORGANIZATION AND LOCALIZATION

In the present Example, variants of KChIP2 and their chromosomal organization were identified using standard techniques. KChIP2 genes are highly conserved at the amino acid level among human, rat, and mouse. Multiple human splice variants were identified by database mining and cDNA library screening. Alternative splicing gives

rise to N-terminal domains that are variable in length, but the core C-terminal domain is sufficient for associating with and modulating Kv4. The human KChIP2 gene spans approximately 18 kb in the q23 region of human chromosome 10 between WI-8488 and WI-6750. This region is syntenic to mouse chromosome 19 between D19Mit40 and D19Mit11. A rat variant discovered by database mining changed the last five amino acids and maintained its ability to associate with and modulate Kv4. Therefore, these multiple variants of KChIP2 appear to function similarly in Kv4 modulation.

#### EXAMPLE 27: KChIP1L FUNCTION AND EXPRESSION

RT-PCR was performed to examine tissue expression of the rat KChIP11 (KChIP1 long) splice variant. PolyA<sup>+</sup> RNA from heart, brain, lung, spleen, liver, skeletal muscle, kidney, and testes were purchased from Clontech. RT-PCR was performed using the One-step RT-PCR kit from Clontech with amplifying 5' primer GGTACCTTCTCGTCCCTGCAGACCAAACAAAG (SEQ ID NO:104) and 3' primer CGGTAAAGGACTTGCAGTTCTCTC (SEQ ID NO:105) with the modifications on PCR condition: 50°C for 1 hour; 94°C for 3 minutes; 50 cycles of 94°C for 30 seconds, 65°C for 30 seconds, and 68°C for 2 minutes. The 5' primer is KChIP1-specific. Both KChIP1 and KChIP11 can be amplified by the same primer set, giving two different sized PCR products that separate into two bands by electrophoresis. A KChIP11-specific band was only observed in brain, indicating it is specifically expressed in the brain. The same reaction also showed a strong KChIP1-specific signal in the brain and a barely visible band in skeletal muscle. No KChIP1 or KChIP11 signals were observed in any other tissues examined. In summary, KChIP11 expression is brain-specific whereas KChIP1 expression is brain predominant with a very low level of expression in skeletal muscle.

The function of KChIP11 in *Xenopus* oocytes was also examined. Kv4.3 cRNA was injected into *Xenopus* oocytes either with or without KChIP11 cRNA. Similar to KChIP1, KChIP11 increased peak amplitude of Kv4.3 from  $15 \pm 4$  to  $55 \pm 7$   $\mu$ A and increased inactivation time constant from  $56 \pm 4$  to  $100 \pm 8$  ms (Table 5). These data demonstrate that KChIP11, like KChIP1, modulates peak amplitude and kinetics of Kv4 current *in vitro*.

Given that the common C-terminal 185 amino acid to both KChIP1 and KChIP11 is responsible for binding to Kv4.3, it is likely that KChIP11 co-associates with Kv4 in the brain. The insertion of extra amino acids in the KChIP11 protein may be important for unknown functions, and the DNA sequence encoding these amino acids may be used as a specific gene marker for detecting cell tissue and/or cell type specific expression of this particular splice variant.

The DNA and protein sequences specific to the KChI1 splice variant are identical between rat and human. So, functional data obtained with KChIP11 molecules from one species also apply to that from the other species.

5 TABLE 5. Modulation of Kv4.3 by KChIP11 and KChIP1N.

Kv4.3 co-expressed with	none	KChIP11	KChIP1	KChIP1N
Inactivation time constant (ms $\pm$ SEM)	56 $\pm$ 4	100 $\pm$ 8	112 $\pm$ 3	1778 $\pm$ 136
Peak amplitude ( $\mu$ A $\pm$ SEM)	15 $\pm$ 4	55 $\pm$ 7	59 $\pm$ 5	18 $\pm$ 3

#### EXAMPLE 28: KChIP1N FUNCTION AND EXPRESSION

10 The expression of rat KChIP1N was examined using the Taqman technique with the probe GGCAAAGAAGCGCGATTTT (SEQ ID NO:106), forward primer TCCCGGGTAGGCAAGCA (SEQ ID NO:107), and reverse primer CCTGCTCAAGCCCAGCACTGCA (SEQ ID NO:108). The probe is specific to KChIP1N. As shown in Figure 68, KChIP1N is predominantly expressed in dorsal root  
15 ganglion (DRG), and at low levels in spinal cord and brain.

The function of KChIP1N in *Xenopus* oocytes was also examined. Kv4.3 cRNA was injected into *Xenopus* oocytes either with or without KChIP1N cRNA. In contrast to KChIP1 and KChIP11, KChIP1N did not affect peak amplitude of Kv4.3 (15  $\pm$  4 vs. 18  $\pm$  3 without or with KChIP1N, Table 5). Surprisingly, KChIP1N caused a much  
20 greater increase of inactivation time constant of Kv4.3 than KChIP1 or KChIP11 (32-fold increase by KChIP1N vs. ~2 fold increase by KChIP1 or KChIP11; Table 5).

The foregoing data demonstrate that KChIP1N modulates Kv4 current *in vitro* in a manner distinct from KChIP1 or KChIP11. First, the increase of the inactivation time constant by KChIP1N was considerably bigger as opposed to the increase mediated by KChIP1 or KChIP11. As a result, KChIP1N was able to change the fast inactivating  
25 Kv4.3 current (nearly completely inactivated within 200 ms) to nearly non-inactivating for a 500 ms second +40 volts pulse. Second, KChIP1N, at the particular concentration tested, did not affect peak amplitude of Kv4. Because all KChIP1 splice variants share the C-terminal 196 amino acids, these data point to an important and distinct function of  
30 the unique 36-amino acid N-terminal domain of KChIP1N.

#### EXAMPLE 29: KChIP2 SPLICE VARIANT FUNCTION

In this Example, the function of KChIP2 splice variants, rat KChIP2I, human KChIP2s, and rat KChIP2C in *Xenopus* oocytes was examined. The results from the  
35 experiments are summarized in the following table.

**TABLE 6.** Modulation of Kv4 current by KChIP2 splice variants.

Kv4.3 co-expressed with	KChIP2l	KChIP2m	KChIP2s	KChIP2C	none
Peak amplitude ( $\mu\text{A} \pm \text{SEM}$ )	$51 \pm 4$	$40 \pm 4$	$44 \pm 3$	$44 \pm 5$	$14 \pm 3$
Inactivation time constant ( $\text{ms} \pm \text{SEM}$ )	$87 \pm 4$	$70 \pm 2$	$90 \pm 3$	$74 \pm 4$	$55 \pm 4$

5 The data demonstrate that these KChIP2 splice variants modulate Kv4 current similar to KChIP2m (Table 6). Since there is an extremely high homology at the amino acid level between the rat and human KChIP2s (>95%), it is believed that the results obtained using KChIP2 molecules from one species will be similar to the results for KChIP2 molecules from other species.

10

#### **EXAMPLE 30: KChIP4 FUNCTION AND EXPRESSION**

Northern analysis was performed to determine the tissue expression of KChIP4. A probe, taken from the 3'UT region of rat KChIP4 (598-909) common to all of the N-terminal splice variants of KChIP4, was used to probe a rat Clontech MTN Northern blot. Among the tissues represented on the Northern blot (heart, brain, lung, spleen, liver, muscle, kidney, and testes), a predominant band of approximately 2.4 kb was observed only in the brain. A faint band with a slightly faster mobility was present in the kidney. Therefore, it is apparent that the N-terminal splice variants of KChIP4 are predominantly expressed in the brain and at lower levels in the kidney.

20 The ability of KChIP4 to associate with Kv4 was also examined using a yeast 2-hybrid assay. The H domain of KChIP4 (the C-terminal 185 amino acids) which is common to all N-terminal splice variants of KChIP4 and homologous to other KChIPs, was expressed as "fish" and the N-terminal domains of Kv4.3, Kv4.2 were expressed as "baits" (Kv4.3N, Kv4.2N, respectively) using standard techniques. KChIP4H associated with Kv4.3N and Kv4.2N, but not with Kv1.1N or other control baits both in a growth assay and in a  $\beta$ -galactosidase assay. These results indicate that KChIP4s bind Kv4 channels in a specific manner.

#### **EXAMPLE 31: FUNCTIONAL ANALYSIS OF KChIP4N2**

30 KChIP4N2, unlike KChIP1, kCHIP2, and KChIP3, showed a dose-dependent effect on the peak amplitude of Kv4.3 when these were co-injected into *Xenopus* oocytes (Table 7). At high concentrations (e.g., 5x dilution of stock), KChIP4N2 suppressed Kv4.3 current amplitude, whereas more diluted concentrations of KChIP4N2 either enhanced or had no effect on Kv4 current amplitude (Table 7)



KChIP4N2, unlike KChIP1, kCHIP2, and KChIP3, also showed a dose-dependent effect on the inactivation kinetics of Kv4.3 when these were co-injected into *Xenopus* oocytes (Table 7). At high concentrations, KChIP4 converted the fast-inactivating Kv4.3 current into an almost non-inactivating current (e.g., at 5x dilution of stock the current curve was too slow to decrease over time to fit and obtain an inactivation time constant). When a more diluted KChIP4N2 cRNA was injected, the inactivation time constants gradually decreased toward the value obtained in the absence of KChIP4N2.

TABLE 7. Modulation of peak amplitude and kinetics of Kv4.3 current by different concentrations of KChIP4N2 in *Xenopus* oocytes.

Kv4.3 co-expressed with KChIP4N2 diluted by factors of (1x=stock)	1x	5x	30x	120x	500x	none
Inactivation time constant (ms $\pm$ SEM)			681 $\pm$ 28	193 $\pm$ 13	84 $\pm$ 5	56 $\pm$ 4
Peak amplitude ( $\mu$ A $\pm$ SEM)	0 $\pm$ 0	4 $\pm$ 1	25 $\pm$ 2	16 $\pm$ 3	9 $\pm$ 4	15 $\pm$ 4

The N-terminal domain of KChIP4N2 is necessary for the observed action of KChIP4N2. Deletion of the N-terminal domain essentially abolished the effects of the wild type KChIP4N2 on the peak amplitude and the inactivation time constant of Kv4.3 (Table 8).

The action of the N-terminal domain of KChIP4N2 seems to be dominant over other KChIP molecules. We made a chimeric molecule, 4N-1H, where the N-terminal domain of KChIP4N2 was fused to the C-terminal 185 amino acid H domain of KChIP1 (KChIP1H, which is homologous to other KChIPs). When co-expressed with Kv4, KChIP1H modulated Kv4 current almost identically to KChIP1, and produced a modulation profile that is quite different from that produced by KChIP4N2 (previous filing, [An F. *et al.* (2000) *Nature* 403:553-556]). However, when co-expressed with Kv4.3, 4N-1H produced a modulation profile almost indistinguishable from that of KChIP4N2 instead of that of KChIP1H or KChIP1 (Table 6). This indicates that the N-terminal domain of KChIP4N2 can function as a module, and its modulatory effect is dominant over the modulatory effects of other KChIPs.

**TABLE 8.** The N-terminal domain of KChIP4N2 is necessary for the effect of KChIP4N2, and is dominant over KChIP1.

Kv4.3 co-expressed with	KChIP4 (30xdilution)	KChIP4H	4N-1H
Inactivation time constant (ms $\pm$ SEM)	681 $\pm$ 28	105 $\pm$ 4	680 $\pm$ 39
Peak amplitude ( $\mu$ A $\pm$ SEM)	25 $\pm$ 2	19 $\pm$ 2	26 $\pm$ 3

Because KChIP4 and other KChIPs associate with the Kv4 N-terminal domain (Kv4N), it is conceivable that these KChIPs bind to the same site on Kv4N. If this is the case, KChIP4N2 and KChIP1 should compete with each other for modulating the Kv4 current when both of them are co-expressed with Kv4. This hypothesis was tested and as indicated in Figure 61, KChIP4N2 and KChIP1 do indeed compete with each other for modulating the Kv4 current. As the concentration of KChIP4 cRNA injected into the *Xenopus* oocytes was held constant whereas the concentration of KChIP1 cRNA was gradually increased, the Kv4.3 current profiles changed from those of KChIP4 to those similar to KChIP1. Reciprocally, as the concentration of KChIP1 cRNA was held constant whereas the concentration of KChIP4 cRNA was gradually increased, the current profiles changed from those of KChIP1 to those similar to KChIP4.

These results indicate that KChIP1 and KChIP4 functionally compete with each other, likely through competitive binding to the same site on Kv4.3N. The results also demonstrate that different combinations of KChIP4N2 and other KChIPs will give rise to currents with hybrid profiles that are quantitatively and qualitatively similar or different from the parental profiles. It is conceivable that KChIP4N2 and other KChIPs are co-expressed in certain cell types *in vivo* (e.g., in the brain). Therefore, depending on the *in vivo* concentrations in a particular cell type, KChIP4N2 and other KChIPs may produce quite different currents even though the pore-forming alpha subunits are the same Kv4 molecules.

The implications of the foregoing observations with respect to KChIP4N2 are many fold. The data indicate that the N-terminal domain carries a dominant modulatory function that can be separated from the functions of the H domain (binding to Kv4 and modulating Kv4 current amplitude and kinetics as described in An *et al.*, *supra*, but in a manner that is different from those of the KChIP4N2's N-terminal domain).

Consequently, it is conceivable that the N-terminal domain of KChIP4N2 interacts with parts of the potassium channel other than the N-terminal domain of Kv4. These other sites on Kv4 are likely important for controlling the movement of potassium ions through the channel, given KChIP4N2's dramatic effect on inactivation kinetics. It is then possible to use the N-terminal domain of KChIP4N2 as a tool for designing and conducting protein/peptide/compound screens using this distinct activity as a read out.

Using these screening assays it is possible to obtain proteins/peptides/compounds that modulate Kv4 activity in a KChIP dependent or independent manner.

As discussed above, KChIP1N and KChIP4N2 share similar Kv4 current modulating characteristics. Both can convert fast-inactivating Kv4 currents into almost non-inactivating currents. Both can have no effect on peak amplitude of Kv4. These are characteristically different from the actions of KChIP1, KChIP2, and KChIP3. Interestingly, when the N-terminal domains of human KChIP1N and monkey KChIP4N2 were aligned (using Megalign, DNA Star), they showed a considerable homology (Figure 62) suggesting the existence of a protein motif that underlies the distinct modulation by KChIP1N and KChIP4N2. In contrast, the N-terminal domains of human/rat KChIP1 and monkey KChIP4N2 were quite divergent (Figure 62).

#### EXAMPLE 32: FUNCTIONAL ANALYSIS OF KChIP4N1 AND KChIP4N3

KChIP4N1 and KChIP4N3 were co-injected with Kv4.3 cRNA into *Xenopus* oocytes. The modulation effects of these proteins on Kv4.3 are summarized in table 9. Both increased the inactivation time constant of Kv4.3. While KChIP4N3 increased the peak amplitude of Kv4.3, KChIP4N1 statistically had no significant (ns) effect on Kv4.3 amplitude.

TABLE 9. Modulation of Kv4 current by KChIP4N1 and KChIP4N3 in *Xenopus* oocytes.

Kv4.3 co-expressed with	KChIP4N1	KChIP4N3	none
Peak amplitude ( $\mu A \pm SEM$ )	$6 \pm 1$ (ns)	$43 \pm 4$	$15 \pm 4$
Inactivation time constant (ms $\pm SEM$ )	$112 \pm 7$	$85 \pm 4$	$56 \pm 4$

#### 25 Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

What is claimed is:

1. An isolated nucleic acid molecule selected from the group consisting of:

- 5                   a) a nucleic acid molecule comprising a nucleotide sequence which is at least 60% identical to the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102, the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316, or a complement thereof;
- 10                   b) a nucleic acid molecule comprising a fragment of at least 583 nucleotides of a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102, the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943,
- 15                   20                   25                   30

98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993,  
98994, or PTA-316, or a complement thereof;

5 c) a nucleic acid molecule which encodes a polypeptide comprising  
an amino acid sequence at least about 60% identical to the amino acid sequence  
of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10,  
SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID  
NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ  
ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38,  
SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID  
10 NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ  
ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85,  
SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID  
NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or  
SEQ ID NO:109 or an amino acid sequence encoded by the DNA insert of the  
15 plasmid deposited with ATCC as Accession Number 98936, 98937, 98938,  
98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948,  
98949, 98950, 98951, 98991, 98993, 98994, or PTA-316;

d) a nucleic acid molecule which encodes a fragment of a  
polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID  
20 NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID  
NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ  
ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32,  
SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID  
NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ  
25 ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78,  
SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID  
NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ  
ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109 or an amino  
acid sequence encoded by the DNA insert of the plasmid deposited with ATCC  
30 as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942,  
98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991,  
98993, 98994, or PTA-316, wherein the fragment comprises at least 15

contiguous amino acid residues of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109 or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316; and

e) a nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109 or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ

ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31,  
 SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID  
 NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ  
 ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75,  
 5 SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID  
 NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ  
 ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID  
 NO:102, or the DNA insert of the plasmid deposited with ATCC as Accession  
 Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944,  
 10 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or  
 PTA-316, under stringent conditions.

2. The isolated nucleic acid molecule of claim 1 which is selected from the group consisting of:

- 15 a) a nucleic acid molecule comprising the nucleotide sequence of  
 SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ  
 ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19,  
 SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID  
 NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ  
 20 ID NO:39, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54,  
 SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID  
 NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ  
 ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90,  
 SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID  
 25 NO:100, or SEQ ID NO:102, or the DNA insert of the plasmid deposited with  
 ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941,  
 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951,  
 98991, 98993, 98994, or PTA-316, or a complement thereof; and
- 30 b) a nucleic acid molecule which encodes a polypeptide comprising  
 the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ  
 ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ  
 ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26,

SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID  
NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ  
ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70,  
SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID  
5 NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ  
ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101,  
SEQ ID NO:103, or SEQ ID NO:109 or an amino acid sequence encoded by the  
DNA insert of the plasmid deposited with ATCC as Accession Number 98936,  
98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946,  
10 98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316.

3. The nucleic acid molecule of claim 1 further comprising vector nucleic  
acid sequences.

15 4. The nucleic acid molecule of claim 1 further comprising nucleic acid  
sequences encoding a heterologous polypeptide.

5. A host cell which contains the nucleic acid molecule of claim 1.

20 6. The host cell of claim 5 which is a mammalian host cell.

7. A non-human mammalian host cell containing the nucleic acid molecule  
of claim 1.

25 8. An isolated polypeptide selected from the group consisting of:

a) a fragment of a polypeptide comprising the amino acid sequence  
of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10,  
SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID  
30 NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ  
ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38,  
SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID



NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or  
5 SEQ ID NO:109 or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:4,  
10 SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID  
15 NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109 or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as  
20 Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316;

b) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ  
25 ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70,  
30 SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101,

SEQ ID NO:103, or SEQ ID NO:109 or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3 SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316 under stringent conditions; and

c) a polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence which is at least 60% identical to a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3 SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98936,

98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946,  
98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316.

d) a polypeptide comprising an amino acid sequence which is at least  
60% identical to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ  
ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ  
ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24,  
SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID  
NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ  
ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59,  
SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID  
NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ  
ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99,  
SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109 or an amino acid  
sequence encoded by the DNA insert of the plasmid deposited with ATCC as  
Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943,  
98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993,  
98994, or PTA-316.

9. The isolated polypeptide of claim 8 comprising the amino acid sequence  
of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ  
ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID  
NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID  
NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID  
NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID  
NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID  
NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID  
NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID  
NO:101, SEQ ID NO:103, or SEQ ID NO:109 or an amino acid sequence encoded by  
the DNA insert of the plasmid deposited with ATCC as Accession Number 98936,  
98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947,  
98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316.

10. The polypeptide of claim 8 further comprising heterologous amino acid sequences.

11. An antibody which selectively binds to a polypeptide of claim 8.

12. A method for producing a polypeptide selected from the group consisting of:

a) a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109 or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316;

b) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109 or an amino acid sequence encoded by the DNA insert of the

plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109 or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316; and

c) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, or SEQ ID NO:109 or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a

- nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3 SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, or SEQ ID NO:102, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316 under stringent conditions;
- comprising culturing the host cell of claim 5 under conditions in which the nucleic acid molecule is expressed.

13. A method for detecting the presence of a polypeptide of claim 8 in a sample comprising:

- a) contacting the sample with a compound which selectively binds to the polypeptide; and
- b) determining whether the compound binds to the polypeptide in the sample to thereby detect the presence of a polypeptide of claim 8 in the sample.

14. The method of claim 13, wherein the compound which binds to the polypeptide is an antibody.

15. A kit comprising a compound which selectively binds to a polypeptide of claim 8 and instructions for use.

16. A method for detecting the presence of a nucleic acid molecule in claim 1 in a sample comprising:

- a) contacting the sample with a nucleic acid probe or primer which selectively hybridizes to the nucleic acid molecule; and
- b) determining whether the nucleic acid probe or primer binds to a nucleic acid molecule in the sample to thereby detect the presence of a nucleic acid molecule of claim 1 in the sample.

17. The method of claim 16, wherein the sample comprises mRNA molecules and is contacted with a nucleic acid probe.

18. A kit comprising a compound which selectively hybridizes to a nucleic acid molecule of claim 1 and instructions for use.

19. A method for identifying a compound which binds to a polypeptide of claim 8 comprising:

- a) contacting the polypeptide, or a cell expressing the polypeptide with a test compound; and
- b) determining whether the polypeptide binds to the test compound.

20. The method of claim 19, wherein the binding of the test compound to the polypeptide is detected by a method selected from the group consisting of:

- a) detection of binding by direct detection of test compound/polypeptide binding;
- b) detection of binding using a competition binding assay; and
- c) detection of binding using an assay for PCIP activity.

21. A method for modulating the activity of a polypeptide of claim 8 comprising contacting the polypeptide or a cell expressing the polypeptide with a compound which binds to the polypeptide in a sufficient concentration to modulate the activity of the polypeptide.

22. A method for identifying a compound which modulates the activity of a polypeptide of claim 8 comprising:

- a) contacting a polypeptide of claim 8 with a test compound; and
- b) determining the effect of the test compound on the activity of the polypeptide to thereby identify a compound which modulates the activity of the polypeptide.

23. A method for identifying a compound capable of treating a disorder characterized by aberrant PCIP nucleic acid expression or PCIP protein activity

comprising assaying the ability of the compound or agent to modulate the expression of the PCIP nucleic acid molecule of claim 1 or the activity of the PCIP polypeptide of claim 8, thereby identifying a compound capable of treating a disorder characterized by aberrant PCIP nucleic acid expression or PCIP protein activity.

24. The method of claim 23, wherein the disorder is a CNS disorder.

25. The method of claim 24, wherein the disorder is epilepsy.

27. The method of claim 24, wherein the disorder is spinocerebellar ataxia.

28. The method of claim 23, wherein the disorder is a cardiovascular disorder.

29. The method of claim 28, wherein the cardiovascular disorder is associated with an abnormal  $I_{to}$  current.

30. A method for determining if a subject is at risk for a disorder characterized by aberrant or abnormal PCIP nucleic acid expression and/or PCIP protein activity comprising detecting, in a sample of cells from the subject, the presence or absence of a genetic lesion, wherein the genetic lesion is characterized by an alteration affecting the integrity of a gene encoding the PCIP polypeptide of claim 8 or misexpression of the PCIP nucleic acid molecule of claim 1.



31. The method of claim 30, wherein the disorder is a CNS disorder.
32. The method of claim 31, wherein the disorder is epilepsy.
- 5 33. The method of claim 31, wherein the disorder is spinocerebellar ataxia.
34. The method of claim 30, wherein the disorder is a cardiovascular  
disorder.
- 10 35. The method of claim 34, wherein the cardiovascular disorder is  
associated with an abnormal  $I_{to}$  current.
- 15 36. A method for identifying a subject suffering from a disorder  
characterized by aberrant or abnormal PCIP nucleic acid expression and/or PCIP protein  
activity comprising obtaining a biological sample from the subject, and detecting in the  
sample, the presence or absence of a genetic lesion, wherein the genetic lesion is  
characterized by an alteration affecting the integrity of a gene encoding the PCIP  
polypeptide of claim 8 or misexpression of the PCIP nucleic acid molecule of claim 1,  
20 thereby identifying a subject suffering from a disorder characterized by aberrant or  
abnormal PCIP nucleic acid expression and/or PCIP protein activity.
37. The method of claim 36, wherein the disorder is a CNS disorder.
- 25 38. The method of claim 37, wherein the disorder is epilepsy.
39. The method of claim 37, wherein the disorder is spinocerebellar ataxia.
40. The method of claim 36, wherein the disorder is a cardiovascular  
30 disorder.

41. The method of claim 40, wherein the cardiovascular disorder is associated with an abnormal  $I_{to}$  current.

42. A method for treating a subject having a potassium channel associated disorder comprising administering to the subject a PCIP polypeptide of claim 8 or portion thereof such that treatment occurs.

43. The method of claim 42, wherein the disorder is a CNS disorder.

44. The method of claim 43, wherein the disorder is epilepsy.

45. The method of claim 43, wherein the disorder is spinocerebellar ataxia.

46. The method of claim 42, wherein the disorder is a cardiovascular disorder.

47. The method of claim 46, wherein the cardiovascular disorder is associated with an abnormal  $I_{to}$  current.

48. A method for treating a subject having a potassium channel associated disorder comprising administering to the subject a nucleic acid encoding a PCIP polypeptide of claim 8 or portion thereof such that treatment occurs.

49. The method of claim 48, wherein the disorder is a CNS disorder.

50. The method of claim 49, wherein the disorder is epilepsy.

51. The method of claim 49, wherein the disorder is spinocerebellar ataxia.

52. The method of claim 48, wherein the disorder is a cardiovascular disorder.

53. The method of claim 52, wherein the cardiovascular disorder is associated with an abnormal  $I_{to}$  current.

54. Use of the compound identified in the method of claim 23 to treat a  
5 potassium channel associated disorder.

## HUMAN IV DNA (CD:225-87)

GAATAGCCCCCTTTCATTCTGAGTCCCTGCATGTGCGGGCTGAAGAAGCAAGCCAGAACCTCTCCTAGCCTCGCCTCCA  
 CGTTTGTGTAATACCAAGCTGCAGGCGAGCTGCGGGCGCTTTCTCTCTCCAAATTCAGAGTAGACAAACCCACGGGGAT  
 TTCCTTCCAGGGTAGGGAGGGCCCGGGCCCGGGGTCCCAACTCGCACTCAAGTCTTCGCTGCCATGGGGCCGTCATGG  
 GCACCTTCTCATCTCTGCAAAACCAACAAAGGCGACCTCGAAGATAAGATTGAAGATGAGCTGGAGATGACCATGGTT  
 TGCCATCGGGCCGAGGGAAGCTGGAGCAGCTCGAGGCCAGACCAACTTCACCAAGAGGGAGCTGCAGGTCTTTTATCGAGG  
 CTTCAAAAATGAGTGGCCCAAGTGGTGGTCAACGAAGACACATCAAGCAGATCTATGCTCAGTTTTCCTCATGGAG  
 ATGCCAGCAGTATGCCCATTAACCTCTTCAATGCCCTTCGACACCACTCAGACAGGCTCCGTGAAGTTCGAGGACTTTGTA  
 ACCGCTCTGTGATTTTATTGAGAGGAACCTGTCCACGAGAACTAAGGTGGACATTTAATTTGTATGACATCAACAAGGA  
 CGGATACATAAACAAGAGAGATGATGGACATTTGTCAAGCCATCTATGACATGATGGGAAATACACATATCCTGTGC  
 TCAAGAGGACACTCCAAGGACGATGTGGACGCTTCTTCCAGAAATGGACAAATAAAGATGGCATCGTAACTTTA  
 GATGAATTTCTTGAATCATGTCCAGGAGGACGACACATCATGAGGTCTCTCCAGCTGTTTCAAAATGTCTGTAACCTGGT  
 GACACTCAGCCATTCACTCTCAGAGACATTTGTACTAAACAACCACTTAACACCCCTGATCTGCCCTTGTCTGATTTTA  
 CACACCAACTCTTGGGACAGAAACACCTTTTACACTTTGGAGAATCTCTCTGCTGAAGACTTCTTTAATGGAACCCAGCAT  
 CATGTGGCTCAGTCTCTGATTGCCAACTCTTCCCTCTTCTTCTTGAGAGAGACAAAGATGAATTTGAGTTTGTGTTG  
 GAAGCATGCTCATCTCTCAGACTGCTGCCCTATGGAAGTCCCTCTGCTTAAGCTTAAACAGTAGTGACACAAAATATGC  
 TGCTTACGTGCCCCCAGCCCACTGCCCTCCAAGTCAGGACAGCCTTGGTGAATCTGGAGAACGAGGACCTGAGCCAGATG  
 CACACCATCTCTGATGGCCCTCCCAACCAATGTGCTCTTCTCTTGGTGGGAAGAAATGAGAGTTATCCAGAACAA  
 ATTAGGATCTGTGTCATGACCAAGATTGGAGAGCCAGCACCTAACATATGTGGGATAGGACTGAATTTAAGCATGACATT  
 GTCTGATGACCCAAACTGCCCCG

## HUMAN IV PROTEIN

MGAVMGTFSSLTQKQRRPSKDKIEDELEMTWVCHRPEGLEQLAQTNFTKRELQVLYRGFKNECPSGVVNEDTFKQIYAQ  
 FFPHGDASTYAHYLFNAFDTTQTGSKFEDFVTALISLLRGTVHEKLRWTFNLYDINKDGYINKEEMMDIVKAIYDMMGK  
 YTPVLKEDTPRQHVDFVFQKMDKNKDGIVTLDEFLESCQEDDNI MRS LQLFQNV M

Fig. 1

RAT 1vN (r1vN) DNA (CD: 339-1037)

GGCACACAACCCCTGGATTCTTCGGAGAATATGCCGTGAGGTGTTGCCAATTATTAGTTCTCTTGGCTAGCAGATGTTTA  
GGGACTGGTtaaGCCTTTGGAGAAATTACCTTAGGAAAACGGGGAAATAAAAGCAAAGATTACCATGAATTGCAAGATT  
CCTAGCAATTGCAAGgtagGAGGAGAGAGGTGGAGGGCGGAGTAGACAGGAGGGAGGGAGAAAGtgaGAGGAAGCTAGGC  
TGGTGGAAATAACCCCTGCAC TTGGAACAGCGGCAAAGAAGCGGATTTTCCAGCTTtaaATGCC TGCCCGGCTTCTGCTT  
GCCTACCCGGGAACGGAGATGTTGACCCAGGGCGAGTCTGAAGGGCTCCAGACCTTGGGGATAGTAGTGGTCCTGTGTTT  
CTCTCTGAACTACTGCACTACCTCGGGCTGATTGACTTGTCTGGATGACAAGATCGAGGATGATCTGGAGATGACCATGG  
TTTGCCATCGGCCTGAGGGACTGGAGCAGCTTGAGGCACAGACGAAC TTCACCAAGAGAGAAGTCAAGTCCCTTACCGG  
GGATTCAAAAACGAGTGCCCCAGTGGTGTGGTTAACGAAGAGACATTCAAGCAGATCTACGCTCAGTTTTTCCCTCATGG  
AGATGCCAGCACATACGCACATTACCTCTTCAATGCCTTCGACACCACCCAGACAGGCTCTGTAAAGTTCGAGGACTTTG  
TGACTGCTCTGTGATTTTACTGAGAGGAACGGTCCATGAAAACTGAGGTGGACGTTTAATTTGTACGACATCAATAAA  
GACGGCTACATAAAACAAAGAGGAGATGATGGACATAGTGAAAGCCATCTATGACATGATGGGGAAATACACCTATCCTGT  
GCTCAAAGAGGACACTCCCAGGCAGCACGTGGACGTCTTCTTCCAGAAATGGATAAAAATAAAGATGGCATTGTAACGT  
TAGACGAATTTCTCGAGTCCTGTCTAGGAGGATGACAACATCATGAGGTCTCTACAGCTGTTCCAAAATGTCATGTAACGT  
AGGACACTGGCCATCCTGCTCTCAGAGACACTGACAAACACCTCAATGCCCTGATCTGCCCTTGTTCAGTTTTACACAT  
CAACTCTCGGGACAGAAATACCTTTTACACTTTTGAAGAATTCTCTGCTGAAGACTTTTACAAAACCTGGCACCGAGTG  
GCTCAGTCTCTGATTGCCAACTCTTCCTCCCTCCTCCTCTTGAGAGGGACGAGCTGAAATCCGAAGTTTGTGTTTGAAGC  
ATGCCCATCTCTCCATGCTGCTGCTGCCCTGTGGAAGGCCCCCTCTGCTTGAGCTTAAACAGTAGTGACAGTTTTCTGCG  
TATACAGATCCCCAACTCACTGCCTCTAAGTCAGGCAGACCTGATCAATCTGAACCAAATGTGCACCATCCTCCGATGG  
CCTCCCAAGCCAAATGTGCCTGCTTCTCTTCTCTGCTGGGAAGAAAGAACGCTCTACAGAGCACTTAGAGCTTACCATGA  
AAATACTGGGAGAGGCAGCACCTAACACATGTAGAAATAGGACTGAATTATTAAGCATGGTGGTATCAGATGATGCAACA  
GCCCATGTCATTTTTTTTTCCAGAGGTAGGGACTAATAATTCTCCCACACTAGCACCTACGATCATAGAACAAGTCTTTT  
AACACATCCAGGAGGGAAACCGCTGCCAGTGGTCTATCCCTTCTCTCCATCCCCTGCTCAAGCCCAGCACTGCATGTCT  
CTCCCGGAAGGTCCAGAATGCCTGTGAAATGCTGTAAC TTTATACCTGTTATAATCAATAAACAGAACTATTTTCGTAC  
AAAAAAAAAAAAAAAAA

Fig. 2

RAT 1vN (r1vN) PROTEIN

MLTQGESEGLQTLGIVVLCSSLKLLHYLGLIDLSDDKIEDDLEMTMVCHRPEGLEQLEAQTNFTKRELQVLYRGFKNEC  
PSGVVNEETFKQIYAQFFPHGDASTYAHYLFNAFDTTQTGSVKFEDFVTALSILLRGTVHEKLRWTFNLYDINKDGYINK  
EEMMDIVKAIYDMMGKYTFVLKEDTPRQHVDVFFQKMDKNKDGIVTLDEFLESCQEDDNIMRSLQLFQNVN

Fig. 2 Continued

MOUSE 1V (CD:477-1127)

CGGCCCCCTGAGATCCAGCCCGAGCGCGGGCGGAGCGGCCGGGTGGCAGCAGGGCGGGCGGGCGGAGCGCAGCTCCCC  
CACCACACGCGCGCGGGCTCGGCAGCCTCGGCCGTGCGGGCACGCCGGCCCCGTGTCCAACATCAGGCAGGCTTTGGGG  
CTCGGGGCTCGGGCTCGGAGAAGCCAGTGGCCCCGGCTGGGTGCCCGCACCGGGGGCGCCTGTCAAGGCTCCCCGCGAGC  
CTCTGGCCCTGGGAGTCAGTGCATGTGCCTGGCTGAAGAAGGCAGCAGCCACGAGCTCCAGGCGCCCCGGCCCCACGTTT  
TCTGAATACCAAGCTGCAGGCGAGCTGCTCGGGGCTTTTTTGTCTTCTCGCTTTTCCTCTCCTCCAATTCAAAGTGGGCA  
ATCCACACCGATTTCTTTTCAGGGGAGGGAAGAGACAGGGCCTGGGGTCCCAAGACGCACACAAGTCTTCGCTGCCATGG  
GGGCCGTGATGGGCATTTCTCCTCCCTGCAGACCAAACAAAGCGACCCCTCTAAAGACAAGATTGAGGATGAGCTAGAG  
ATGACCATGGTTTGCCACCGGCCTGAGGGACTGGAGCAGCTTGAGGCACAGACGAACTTCACCAAGAGAGAACTGCAAGT  
CTTGTAACCGGGATTCAAAAACGAGTGCCCTAGCGGTGTGGTCAATGAAGAAACATTCAAGCAGATCTACGCTCAGTTTT  
TCCCTCACGGAGATGCCAGCACATATGCACATTACCTCTTCAATGCCTTCGACACCACCCAGACAGGCTCTGTAAAGTTC  
GAGGACTTTGTGACTGCTCTGTGATTTTACTGAGAGGGACAGTCCATGAAAACTAAGGTGGACGTTTTAATTTGTATGA  
CATCAATAAAGACGGCTACATAAAACAAAGAGGAGATGATGGACATAGTCAAAGCCATCTATGACATGATGGGGAAATACA  
CCTATCCTGTGCTCAAAGAGGACACTCCAGGCAGCATGTGGATGTCTTCTTCCAGAAAATGGATAAAAATAAGATGGC  
ATTGTAACGTTAGATGAATTTCTTGAATCATGTCAGGAGGATGACAACATCATGAGATCTCTACAGCTGTTCCAAAATGT  
CATGTAAGTGAAGACTGGCCATTCTGCTCTCAGAGACACTGACAAACACCTTAATGCCCTGATCTGCCCTTGTTCCAA  
TTTTACACACCAACTCTTGGGACAGAAATACCTTTTACACTTTTGAAGAATTCTCTGCTGAAGACTTTCTACAAAACCTG  
GCACCACGTGGCTCTGTCTCTGAGGGACGAGCGGAGATCCGACTTTGTTTTGGAAGCATGCCCATCTCTTCATGCTGCTG  
CCCTGTGGAAGGCCCTCTGCTTGAGCTTAATCAATAGTGACAGTTTTATGCTTACACATATCCCCAACTCACTGCCTC  
CAAGTCAGGCAGACTCTGATGAATCTGAGCCAAATGTGCACCATCCTCCGATGGCCTCCCAAGCCAATGTGCCTGCTTCT  
CTTCCTCTGGTGGGAAGAAAGAGTGTCTACGGAACAATTAGAGCTTACCATGAAAATATTGGGAGAGGCAGCACCTAAC  
ACATGTAGAATAGGACTGAATTATTAAGCATGGTGATATCAGATGATGCAAATTGCCCATGTCATTTTTTTCAAAGGTAG  
GGACAAATGATTCTCCACACTAGCACCTGTGGTCATAGAGCAAGTCTCTTAACATGCCCAGAAGGGGAACCACTGTCCA  
GTGGTCTATCCCTCCTCTCCATCCCTGCTCAAACCCAGCACTGCATGTCCCTCCAAGAAGGTCCAGAATGCCTGCCAAA  
CGCTGTACTTTTATACCCTGTTCTAATCAATAAACAGAACTATTTTCGTAAAAA

MOUSE 1V PROTEIN

MGAVMGTFSSLQTKQRRPSKDKIEDELEMTMVCHRPEGLEQLEAQTNFTKRELQVLYRGFKNECPSGVVNEETFQIYAO  
FFPHGDASTYAHYLFNAFDTTQTSVKFEDFVTALSILLRGTVHEKLRWTFNLYDINKDGYINKEEMMDIVKAIYDMMGK  
VTYPVLKEDTFRQHVDVFFQKMDKNKDGIVTLDEFLESCQEDDNIMRSLQLFQNM

Fig. 3

## RAT 1VL DNA (CD:31-714)

GTCCCAAGTCGCACACAAGTCTTCGCTGCCATGGGGGCCGTCATGGGTACCTTCTCGTCCCTGCAGACCAACAAAGGCG  
ACCCCTCTAAAGACATCGCCTGGTGGTATTACCAGTATCAGAGAGACAAGATCGAGGATGATCTGGAGATGACCATGGTTT  
GCCATCGGCCTGAGGGACTGGAGCAGCTTGAGGCACAGACGAACCTCACCAAGAGAGAACTGCAAGTCCTTTACCGGGGA  
TTCAAAAACGAGTGCCCCAGTGGTGTGGTTAACGAAGAGACATTCAAGCAGATCTACGCTCAGTTTTTCCCTCATGGAGA  
TGCCAGCACATACGCACATTACCTCTTCAATGCCCTTCGACACCAACCCAGACAGGCTCTGTAAAGTTCGAGGACTTTGTGA  
CTGCTCTGTCGATTTTACTGAGAGGAACGGTCCATGAAAACTGAGGTGGACGTTTAATTTGTACGACATCAATAAGAC  
GGCTACATAAAACAAAGAGGAGATGATGGACATAGTGAAAGCCATCTATGACATGATGGGGAAATACACCTATCCTGTGCT  
CAAAGAGGACACTCCCAGGCAGCACGTGGACGTCTTCTTCCAGAAAATGGATAAAAAATAAGATGGCATTGTAACGTTAG  
ACGAATTTCTCGAGTCTCTGTCAGGAGGATGACAACATCATGAGGTCTCTACAGCTGTTCCAAAATGTCATGTAACGAGG  
ACACTGGCCATCCTGCTCTCAGAGACACTGACAAACACCTCAATGCCCTGATCTGCCCTTGTTCCAGTTTTACACATCAA  
CTCTCGGGACAGAAATACCTTTTACACTTTGGAAGAATTCTCTGCTGAAGACTTTCTACAAAACCTGGCACCGCGTGGCT  
CAGTCTCTGATTGCCAACTCTTCTCCTCCCTCCTCTCTTGAGAGGGACGAGCTGAAATCCGAAGTTTGTGTTTGGAGCATG  
CCCATCTCTCCATGCTGCTGCTGCCCTGTGGAAGGCCCTCTGCTTGAGCTTAAACAGTAGTGACACAGTTTTCTGCGTAT  
ACAGATCCCCAACTCACTGCCTCTAAGTCAGGCAGACCTGATCAATCTGAACCAAATGTGCACCATCCTCCGATGGCCT  
CCCAAGCCAATGTGCCTGCTTCTCTTCTCTGGTGGGAAGAAAGAACGCTCTACAGAGCACTTAGAGCTTACCATGAAAA  
TACTGGGAGAGGCAGCACCTAACACATGTAGAATAGGACTGAATTATTAAGCATGGTGGTATCAGATGATGCAAACAGCC  
CATGTCAATTTTTTTTCCAGAGGTAGGGACTAATAATTCTCCCACACTAGCACCTACGATCATAGAACAAGTCTTTTAACA  
CATCCAGGAGGGAAACCGCTGCCAGTGGTCTATCCCTTCTCTCCATCCCTGCTCAAGCCCAGCACTGCATGTCTCTCC  
CGGAAGGTCCAGAATGCCTGTGAAATGCTGTAACTTTTATACCTGTTATAATCAATAAACAGAACTATTTTCGTACAAAA  
AAAAAAAAAAAAA

## RAT 1VL PROTEIN

MGAVMGTFSSLQTKQRRPSKDIAWYYQYQDKIEDDLEMTMVCHRPEGLEQLEAQTNFTKRELQVLYRGFKNECPSGVV  
NEETFKQIYAQFFPHGDASTYAHYLFNAFDTTQTGSVKFEDFVTALSILLRGTVHEKLRWTFNLYDINKDGYINKKEEMD  
IVKAIYDMMGKYTYPVLKEDTPRQHVDFVFQMDRKNKGIVTLDEFLESCQEDDNIMRSLQLFQNVH

Fig. 4



## MOUSE 1VL DNA (CD:77-760)

ATCCACACCGATTTCCTTTTCAGGGGAGGGAAGAGACAGGGCCTGGGGTCCCAAGACGCACACAAGTCTTCGCTGCCATGG  
GGGCCGTCATGGGCACTTCTCCTCCCTGCAGACCAAACAAGGCGACCCTCTAAAGACATCGCCTGGTGGTATTACCAG  
TATCAGAGAGACAAGATTGAGGATGAGCTAGAGATGACCATGGTTTGGCACCGGCCTGAGGGACTGGAGCAGCTTGAGGC  
ACAGACGAACTTCACCAAGAGAGAACTGCAAGTCTTGTACCGGGGATTCAAAAACGAGTGCCCTAGCGGTGTGGTCAATG  
AAGAAACATTCAAGCAGATCTACGCTCAGTTTTTCCCTCACGGAGATGCCAGCACATATGCACATTACCTCTTCAATGCC  
TTCGACACCAACCCAGACAGGCTCTGTAAAGTTCGAGGACTTTGTGACTGCTCTGTGCGATTTTACTGAGAGGGACAGTCCA  
TGAAAAACTAAGGTGGACGTTTAATTTGTATGACATCAATAAAGACGGCTACATAAACAAAGAGGAGATGATGGACATAG  
TCAAAGCCATCTATGACATGATGGGGAAATACACCTATCCTGTGCTCAAAGAGGACACTCCCAGGCAGCATGTGGATGTC  
TTCTTCCAGAAAATGGATAAAAAATAAGATGGCATTTGTAACGTTAGATGAATTTCTTGAATCATGTCAGGAGGATGACAA  
CATCATGAGATCTCTACAGCTGTTCCAAAATGTCATGTAAGTGGAGACTGGCCATTCTGCTCTCAGAGACACTGACAA  
ACACCTTAATGCCCTGATCTGCCCTTGTTCCTCAATTTTACACACCAACTCTTGGGACAGAAATACCTTTTACACTTTGGAA  
GAATTCTCTGCTGAAGACTTTCTACAAAACCTGGCACCACGTGGCTCTGTCTCTGAGGGACGAGCGGAGATCCGACTTTG  
TTTTTGAAGCATGCCCATCTCTTCATGCTGCTGCCCTGTGGAAGGCCCTCTGCTTGAGCTTAATCAATAGTGCACAGTT  
TTATGCTTACACATATCCCCAACTCACTGCCCTCCAAAGTCAGGCAGACTCTGATGAATCTGAGCCAAATGTGCACCATCCT  
CCGATGGCCTCCCAAGCCAATGTGCCTGCTTCTCTTCCCTCTGGTGGGAAGAAAAGAGTGTCTACGGAACAATTAGAGCTT  
ACCATGAAAATATTGGGAGAGGCAGCACCTAACACATGTAGAATAGGACTGAATTATTAAGCATGGTGATATCAGATGAT  
GCAAAATTGCCCATGTCAATTTTTTTCAAAGGTAGGGACAAATGATTCTCCCACTAGCACCTGTGGTCATAGAGCAAGTC  
TCTTAACATGCCCAGAAGGGGAACCACTGTCCAGTGGTCTATCCCTCCTCTCCATCCCCTGCTCAAACCCAGCACTGCAT  
GTCCCTCCAAGAAGGTCCAGAATGCCTGCGAAACGCTGTACTTTTATACCTGTTCTAATCAATAAACAGAACTATTTCC  
TACAAAAAAAAAAAAAAAAA

## MOUSE 1VL PROTEIN

MGAVMGTFSLSLQTKQRRPSKDIAWYYQYQRDKIEDELEMTMVCHRPEGLEQLEAQTNFTKRELQVLYRGFKNECPSGVV  
NEETFQKIYAQFFPHGDASTYAHYLFNAFDTTQTGSKVFEDFVTALSI LLRGTVHEKLRWTFNLYDINKDGYINKEEMMD  
IVKAIYDMMGKRYTYPVLKEDTPRQHVVDVFFQKMDRNKDGIVTLDEFLESCQEDDNIMRSLQLFQNVW

Fig. 5

RAT 1VN DNA (FIRST-PASS,PARTIAL;CD: 345-955)

GTCCGGGCACACAACCCCTGGATTCTTCGGAGAATATGCCGTGACGGTGTTGCCAATTATTAGTTCTCTTGGCTAGCAGA  
TGTTTAGGGACTGGTTAAGCCTTTGGAGAAATTACCTTAGGAAAACGGGGAAATAAAAGCAAAGATTACCATGAATTGCA  
AGATTACCTAGCAATTGCAAGGTAGGAGGAGAGAGGTGGAGGGCGGAGTAGACAGGAGGGAGGGAGAAAGTGAGAGGAAG  
CTAGGCTGGTGGAAATAACCCTGCACTTGGAACAGCGGCAAAGAAGCGCGATTTTCCAGCTTTAAATGCCTGCCC GCGTT  
CTGCTTGCCTACCCGGGAACGGAGATGTTGACCCAGGGCGAGTCTGAAGGGCTCCAGACCTTGGGGATAGTAGTGGTCCT  
GTGTTCTCTCTGAACTACTGCACTACCTCGGGCTGATTGACTTGTTCGGATGACAAGATCGAGGATGATCTGGAGATGA  
CCATGGTTTGCCATCGGCCTGAGGGACTGGAGCAGCTTGAGGCACAGACGAACTTCACCAAGAGAGAACTGCAAGTCCTT  
TACCGGGGATTCAAAAACGAGTGCCCCAGTGGTGTGGTTAACGAAGAGACATTCAAGCNGATCTACGCTCAGTTTTTCCC  
TCATGGAGATGCCAGCACATACGCACATTACCTCTTCAATGCCTTCGACACCACCCAGACAGGCTCTGTAAAGTTCGAGG  
ACTTTGTGACTGCTCTGTTCGATTTTACTGAGAGGAACGGTCCATGAAAACCTGAAGTGGACGTTTAAATTTGTACGACATC  
AATAAAGACGGCTACATAAAACAAAGAGGAGATGATGGACATAGTGAAAGCCATCTATGACATGATGGGGAAATACACCTA  
TCTTGTGCTCAAAGAGGACACTTCCAGGCAGCACGTGGACGTCTTCTTCCAGAAAATGGATAAAAATAAAGATGG

RAT 1VN PROTEIN (PARTIAL)

MLTQGESEGLQTLGIVVVLCSLLKLLHYLGLIDLSDDKIEDDLEMTMVCHRPEGLEQLAQTNFTKRELVLYRGFKNEC  
PSGVVNEETFXXIYAQFFPHGDASTYAHYLFNAFDTTQTGSVKFEDFVTALSILLRGTVHEKLEKWTFFNLYDINKDGYINK  
EEMMDIVKAIYDMMGKYTYLVLEKEDTSRQHVDVFFQKMDKNEK

Fig. 6

HUMAN 9QL DNA (CD:207-1019)

CTCACCTGCTGCTAGTGTTCCTCTCTGCTCCAGGACCTCCGGGTAGACCTCAGACCCCGGGCCCATTCCCAGACTCA  
GCCTCAGCCCGGACTTCCCCAGCCCCGACAGCACAGTAGGCCGCCAGGGGGCGCGTGTGAGCGCCCTATCCCGGCCACC  
CGGCGCCCCCTCCACGGCCCCGGGGGAGCGGGGCCCGGGGGCCATGCGGGGCCAGGGCCCAAGGAGAGTTTGTCCG  
ATTCCCAGACCTGGACGGCTCCTACGACCAGCTCACGGGCCACCCTCCAGGGCCCACTAAAAAGCGCTGAAGCAGCGA  
TTCTCAAGCTGCTGCCGTGCTGCGGGCCCCAAGCCCTGCCCTCAGTCAGTGAAACATTAGCCGCCCCAGCCTCCCTCCG  
CCCCACAGACCCCGCTGCTGGACCCAGACAGCGTGGACGATGAATTTGAATTGTCCACCGTGTGTCACCGGCTGAGG  
GTCTGGAGCAGCTGCAGGAGCAAACCAATTACGCGCAAGGAGTTGCAGGTCTGTACCGGGCTTCAAGAACGAATGT  
CCCAGCGGAATTGTCAATGAGGAGAACTTCAAGCAGATTTACTCCCAGTTCTTTCTCAAGGAGACTCCAGCACCTATGC  
CACTTTTCTCTTCAATGCCTTTGACACCAACCATGATGGCTCGGTCAGTTTTGAGGACTTTGTGGCTGGTTTGTCCGTGA  
TTCTTCGGGGAAGCTGTAGATGACAGGCTTAATTGGGCCCTTCAACCTGTATGACCTTAACAAGGACGGCTGCATCACCAAG  
GAGGAAATGCTTGACATCATGAAGTCCATCTATGACATGATGGGCAAGTACACGTACCTGCACTCCGGGAGGAGGCCCC  
AAGGGAACACGTGGAGAGCTTCTTCCAGAAGATGGACAGAAACAAGGATGGTGTGGTGACCATTGAGGAATTCATTGAGT  
CTTGTCAAAAGGATGAGAACATCATGAGGTCCATGCAGCTCTTTGACAAATGTCATCTAGCCCCCAGGAGAGGGGGTCACT  
GTTTCTCGGGGGACCATGCTCTAACCCTAGTCCAGGCGGACCTCACCCCTCTCTTCCCAGGTCTATCCTCATCCTACGC  
CTCCCTGGGGGCTGGAGGGATCCAAGAGCTTGGGGATTCACTAGTCCAGATCTCTGGAGCTGAAGGGGCCAGAGAGTGGG  
CAGAGTGCATCTCGGGGGGTGTTCCCAACTCCCACCAGCTCTCACCCCTTCTCTGCTGACACCCAGTGTTGAGAGTGCC  
CCTCCTGTAGGAATTGAGCGGTTCCCCACCTCCTACCTACTCTAGAAACACACTAGAGCGATGTCTCCTGCTATGGTGC  
TTCCCCCATCCCTGACCTCATAAACATTTCCCTAAGACTCCCTCTCAGAGAGAATGCTCCATTCTTGGCACTGGCTGG  
CTTCTCAGACCAGCCATTGAGAGCCCTGTGGGAGGGGGACAAGAATGTATAGGGAGAAATCTTGGGCCTGAGTCAATGGA  
TAGGTCTTAGGAGGTGGGTGGGGTTGAGAATAGAAGGGCCTGGACAGATTATGATTGCTCAGGCATACCAGGTTATAGCT  
CCAAGTTCCACAGGTCTGCTACCACAGGCCATCAAAATATAAGTTTCCAGGCTTTGCAGAAGACCTTGTCTCCTTAGAAA  
TGCCCCAGAAATTTCCACACCCCTCCTCGGTATCCATGGAGAGCCTGGGGCCAGATATCTGGCTCATCTCTGGCATTGCT  
TCTCTCCTTCTCTCTGTCATGTGTTGGTGGTGGTGTGGTGGGGGAATGTGGATGGGGGATGCTCTGGCTGATGCCTGC  
CAAAATTTTCATCCCACCCCTCCTTGCTTATCGTCCCTGTTTTGAGGGCTATGACTTGAGTTTTTGTTCCTCATGTTCTTA  
TAGACTTGGGACCTTCTGAACTTGGGGCCTATCACTCCCCACAGTGGATGCCTTAGAAGGGAGAGGGAAGGAGGGAGGC  
AGGCATAGC

Fig. 7

## HUMAN 9QL PROTEIN

MRGQGRKESLSDSRDL DGSYDQLTGHPGPTKKALKQRFLKLLPCCGPQALPSVSETLAAPASLRPHRPRLLDPDSVDDE  
FELSTVCHRPEGLEQLQEQTFRKELQVLYRGFKNECP SGIVNEENFKQIYSQFFPQGDSSTYATFLFNAFDTNHDGSV  
SFEDFVAGLSVILRGTVDDRLN WAFNLYDLNKDGCITKEEMLDIMKSIYDMMGKYTYPALREEAPREHVESFFQMRNK  
DGVVTIEEFIESCQK DENIMRSMQLFDNVI

Fig. 7 Continued

## RAT 9QL DNA (PARTIAL;CD:2-775)

CCGAGATCTGGACGGCTCCTATGACCAGCTTACGGGCCACCCTCCAGGGCCCAGTAAAAAGCCCTGAAGCAGCGTTTCC  
TCAAGCTGCTGCCGTGCTGCGGGCCCCAAGCCCTGCCCTCAGTCAGTGAAACATTAGCTGCCCCAGCCTCCCTCCGCCCC  
CACAGACCCCGCCCGCTGGACCCAGACAGCGTAGAGGATGAGTTTGAATTATCCACGGTGTGTCACCGACCTGAGGGCCT  
GGAACAACCTCCAGGAACAGACCAAGTTCACACGCAGAGAGCTGCAGGTCTGTACCGAGGCTTCAAGAACGAATGCCCCA  
GTGGGATTGTCAACGAGGAGAACTTCAAGCAGATTTATTCTCAGTTCTTTCCCCAAGGAGACTCCAGCAACTATGCTACT  
TTTCTCTTCAATGCCTTTGACACCAACCACGATGGCTCTGTCAAGTTTGAAGACTTTGTGGCTGGTTTGTCCGGTGATTCT  
TCGGGGGACCATAGATGATAGACTGAGCTGGGCTTTCAACTTATATGACCTCAACAAGGACGGCTGTATCACAAAGGAGG  
AAATGCTTGACATTATGAAGTCCATCTATGACATGATGGGCAAGTACACATACCCTGCCCTCCGGGAGGAGGCCCAAGA  
GAACACGTGGAGAGCTTCTTCCAGAAGATGGACAGGAACAAGGACGGCGTGGTGACCATCGAGGAATTCATCGAGTCTTG  
TCAACAGGACGAGAACATCATGAGGTCCATGCAGCTCTTTGATAATGTCATCTAGCTCCCCAGGAGAGGGGTTAGTGTG  
TCCTAGGGTGACCAGGCTGTAGTCCTAGTCCAGACGAACCTAACCCTCTCTCTCCAGGCCTGTCTCATCTTACCTGTAC  
CCTGGGGGCTGTAGGGATTCAATATCCTGGGGCTTCAGTAGTCCAGATCCCTGAGCTAAGTCACAAAAGTAGGCAAGAGT  
AGGCAAGCTAAATCTGGGGGCTTCCCAACCCCGACAGCTCTCACCCTTCTCAACTGATACCTAGTGCTGAGGACACCC  
CTGGTGTAGGGACCAAGTGGTTCTCCACCTTCTAGTCCCACTCTAGAAACCACATTAGACAGAAGGTCTCCTGCTATGGT  
GCTTTCCCATCCCTAATCTCTTAGATTTTCTCAAGACTCCCTTCTCAGAGAACAGCTCTGTCCATGTCCCAGCTGG  
GGACATGGACAGAGCGTGTCTCTAGTTCTAGATCGCGAGCGGCCGC

## RAT 9QL PROTEIN (PARTIAL)

RDLDGSYDQLTGHPGPSKKALKQRFLLPCCGPQALPSVSETLAAPASLRPHRPRPLDPDSVEDEFELSTVCHRPEGL  
EQLQEQTKFTRRELQVLYRGFKNECPSGIVNEENFKQIYSQFFPQGDSSNYATFLFNAFDTHDGSVSFEDFVAGLSVIL  
RGTIDDRLSWAFNLYDLNKGDCITKEEMLDIMKSIYDMMGKYTYPALREEAPREHVESFFQKMDRNRKDGVTTFEEFIESC  
QQDENIMRSMQLFDNVI

Fig. 8

## MOUSE 9QL DNA (CD:181-993)

CGGGACTCTGAGGTGGGCCCTAAATCCAGCGCTCCCCAGAGAAAAGCCTTGCCAGCCCCTACTCCCGCCCCCAGCCCC  
AGCAGGTGCGTGCGCCGCCAGGGGGCACTGTGTGAGCGCCCTATCTGGCCACCCGGCGCCCCCTCCACGGCCAGGGC  
GGAGCGGGGCGCCGGGGGCCATGCGGGGCCAAGGCCGAAAGGAGAGTTTGTCCGAATCCCGAGATTGGACGGCTCCTAT  
GACCAGCTTACGGGCCACCTCCAGGGCCAGTAAAAAGCCCTGAAGCAGCGTTTCCTCAAGCTGCTGCCGTGCTGCGG  
GCCCCAAGCCCTGCCCTCAGTCAGTGAAACATTAGCTGCCCCAGCCTCCCTCCGCCCCACAGACCCCGCCGCTGGACC  
CAGACAGCGTGGAGGATGAGTTTGAACATACACGGTGTGCCACCGGCTGAGGGTCTGGAACAACCTCCAGGAACAAACC  
AAGTTCACACGCAGAGAGTTGCAGGTCTGTACAGAGGCTTCAAGAACGAATGTCCAGCGGAATTGTCAACGAGGAGAA  
CTTCAAGCAAATTTATTCTCAGTTCTTTCCCCAAGGAGACTCCAGCAACTACGCTACTTTTCTCTTCAATGCCTTTGACA  
CCAACCATGATGGCTCTGTGAGTTTGTGAGGACTTGTGGCTGGTTTGTGAGTGATTCTTCGGGGAACCATAGATGATAGA  
CTGAACTGGGCTTTCAACTTATATGACCTCAACAAGGATGGCTGTATCACGAAGGAGGAAATGCTCGACATCATGAAGTC  
CATCTATGACATGATGGGCAAGTACACCTACCCTGCCCTCCGGGAGGAGGCCCCGAGGGAACACGTGGAGAGCTTCTTCC  
AGAAGATGGACAGAAAACAAGGACGGCGTGGTGACCATTGAGGAATTCATTGAGTCTTGTCAACAGGACGAGAATCATG  
AGGTCCATGCAACTCTTTGATAATGTATCTAGCTCCCCAGGGAGAGGGGTAGTGTGTCCAGGGTAACCATGCTGTAG  
CCCTAGTCCAGGCAAACTAACCCCTCCTCTCCCCGGGTCTGTCTCATCTACCTGTACCCTGGGGGTGTAGGGATTCA  
ACATCCTGGCGCTTCAGTAGTCCAGATCCCTGAGCTAAGTGGCGAGAGTAGGCAAGCTAAGTCTTTGGAGGGTGGGTGGG  
GGCGCGCAGATTTCCAAACCCCGACGACTCTCACCCCTTTCTCGACTGATACCCAGTGTGAGGCTACCCCTGGTGTGCG  
GAACGACCAAGTGGTTCTCTGCCTCCCCAGCCACTCTAGAGACCCACACTAGACGGGAATATCTCTGCTATGGTGCT  
TTCCCCATCCCTGACCGCAGATTTTCTCTTAAGACTCCCTTCTCAGAGAATATGCTTTTGTCCCTGTCCCTGGCTGGC  
TTTTCAGCCTAGCCTTTGAGGACCCTGTGGGAGGGGAGAATAAGAAAGCAGACAAAATCTTGGCCCTGAGCCAGTGTTA  
GGTCTTAGGAATCAGGCTGGAGTGGAGACCAGAAAGCCTGGGCAGGCTATGAGAGCCCCAGGTTGGCTGTACCGCCAG  
GTTCCACAGGGCTGCTGCTCTGGGTGAGCAGAGTATGAGTTTCCAGACTTTCCAGAAGGCCTTATGTCTTAGCAATGTC  
CCAGAAATTCACCATACACTTCTCAGTGTCTTAGGATCCAGATGTCCGGTCCATCCCTGAAACCTCTCCCTCCTCTTGC  
TCCTATGGTGGGAGTGGTGGCCAGGGGACGATGAGTGAGCCGGTGTCTGGATGATGCCGTGTCAAGGTCCACCTACCCT  
CCGGCTGTCAAGCCGTCTGTTGACCTGTTTGAATCTCCATGACCCCTGTCTAGATGTAGAGGTGTGGAGTGAGTCTAG  
TGGCAGCCTTAGGGGAATGGGAAGAAGAGAGGGGCACTCCATCTGAACCCAGTGTGGGGGCATCCATTGGAATCTTTC  
CTGGCTCCCCACAATGCCCTAGGATCCTCTAGGGTCCCCACCCCACTCTTTAGTCTACCCAGAGATGCTCCAGAGCTCA  
CCTAGAGGGCAGGGACCATAGGATCCAGGTCCAACCTGTCTATCAGCATCCGGCCATGCTGCTGCTGCTTATTAATAAAC  
TGCTTGTCGTTTACGCGCCCTTCCCAGTCAGCCAGGGTCTGAGGGGAAGGCCCCACTTTCCCGCCTCCTGTGACATT  
GTTGACTGCTTTGCATTTTGGGCTCTTCTACCTATATTTGTATAATAAGAAAGACACCAGATCCAATAAACACATGGC  
TATGCACAAAAA

## MOUSE 9QL PROTEIN

MRGQGRKESLSERDLGSDYDLTGHPGPSKKALKQRFLKLLPCCGPQALPSVSETLAAPASLRPHRPRPLDPDSVEDE  
FELSTVCHRPEGLEQLQEQTFRRELQVLYRGFKNECPGIVNEENFKQIYSQFFPQGDSSNYATFLFNAFDTNHDSV  
SFEDFVAGLSVILRGITIDRLNWFNLYDLNKGDCITKEMLDIMKSIYDMMGKYTYPALREEAPREHVESFFQKMDRNK  
DGVVTIEEFIESCQDENIMRSMQLFDNVI

Fig. 9

HUMAN 9QM DNA (CD:207-965)

CTCACCTGCTGCCTAGTGTTCCCTCTCCTGCTCCAGGACCTCCGGGTAGACCTCAGACCCCGGGCCCATTTCCAGACTCA  
GCCTCAGCCCGGACTTCCCCAGCCCCGACAGCACAGTAGGCCCGCCAGGGGGCGCCGTGTGAGCGCCCTATCCCGGCCACC  
CGGCGCCCCCTCCACGGCCCCGGCGGGAGCGGGGCGCCGGGGGCCATGCGGGGCCAGGGCCGCAAGGAGAGTTTGTCCG  
ATTCCCAGACCTGGACGGCTCCTACGACCAGCTCACGGGCCACCCTCCAGGGCCCACTAAAAAGCGCTGAAGCAGCGA  
TTCTCAAGCTGCTGCCGTGCTGCGGGCCCCAAGCCCTGCCCTCAGTCAGTGAAAACAGCGTGGACGATGAATTTGAATT  
GTCCACCGTGTGTACCGGCCCTGAGGGTCTGGAGCAGCTGCAGGAGCAAACCAAATTCACGCGCAAGGAGTTGCAGGTCC  
TGTAACGGGGCTTCAAGAACGAATGTCCCAGCGGAATTGTCAATGAGGAGAACTTCAAGCAGATTTACTCCCAGTTCTTT  
CCTCAAGGAGACTCCAGCACCTATGCCACTTTTCTCTTCAATGCCTTTGACACCAACCATGATGGCTCGGTCACTTTTGA  
GGACTTTGTGGCTGGTTTGTCCGTGATTCTTCGGGGAAGTGTAGATGACAGGCTTAATTGGGCCCTCAACCTGTATGACC  
TTAACAAGGACGGCTGCATCACCAAGGAGGAAATGCTTGACATCATGAAGTCCATCTATGACATGATGGGCAAGTACACG  
TACCCTGCACCTCCGGGAGGAGGCCCCAAGGGAACACGTGGAGAGCTTCTTCCAGAAGATGGACAGAAACAAGGATGGTGT  
GGTGACCATTGAGGAATTCATTGAGTCTTGTCAAAAAGGATGAGAACATCATGAGGTCCATGCAGCTCTTTGACAATGTCA  
TCTAGCCCCCAGGAGAGGGGGTCACTGTTTCTCGGGGGACCATGCTCTAACCCTAGTCCAGGCGGACCTCACCTTCTC  
TTCCCAGGTCTATCCTCATCTACGCCCTCCCTGGGGGCTGGAGGGATCCAAGAGCTTGGGGATTCACTAGTCCAGATCTC  
TGGAGCTGAAGGGGCCAGAGAGTGGGCAGAGTGCATCTCGGGGGGTGTTCCCAACTCCCACCAGCTCTCACCCCTTCTC  
GCCTGACACCCAGTGTGAGAGTGCCCTCCTGTAGGAATTGAGCGGTTCCCCACCTCCTACCTACTCTAGAAACACAC  
TAGAGCGATGTCTCCTGCTATGGTGCTTCCCCCATCCCTGACCTCATAAACATTTCCCCTAAGACTCCCCCTCTCAGAGAG  
AATGCTCCATTCTTGGCACTGGCTGGCTTCTCAGACCAGCCATTGAGAGCCCTGTGGGAGGGGGACAAGAATGTATAGGG  
AGAAATCTTGGGCCTGAGTCAATGGATAGGTCTTAGGAGGTGGGTGGGGTTGAGAATAGAAGGGCCTGGACAGATTATGA  
TTGCTCAGGCATACCAGGTTATAGCTCCAAGTTCACAGGTCTGCTACCACAGGCCATCAAAATATAAGTTTCCAGGCTT  
TGCAGAAGACCTTGTCTCCTTAGAAATGCCCCAGAAATTTCCACACCCCTCCTCGGTATCCATGGAGAGCCTGGGGCCAG  
ATATCTGGCTCATCTCTGGCATTGCTTCTCTCCTTCTCTCTGTCATGTGTTGGTGGTGGTGTGGTGGGGGAATGTGGA  
TGGGGGATGTCTGGCTGATGCTGCCAAAATTTTCATCCACCCCTCCTTGCTTATCGTCCCTGTTTTGAGGGCTATGACT  
TGAGTTTTTGTTCCTCATGTCTCTATAGACTTGGGACCTTCTGAACTTGGGGCCTATCACTCCCCACAGTGGATGCCT  
TAGAAGGGAGAGGGAAGGAGGGAGGCAGGCATAGC

Fig. 10

## HUMAN 9QM PROTEIN

MRGQGRKESLSDSRDLGSDYDQLTGHPGPTKKALKQRFLKLLPCCGPQALPSVSENSVDDEFELSTVCHRPEGLEQLQE  
QTKFTRKELQVLYRGFKNECPGIVNEENFKQIYSQFFPQGDSSTYATFLNAFDTNHDGSVSFEDFVAGLSVILRGTV  
DRLNWAFNLYDLNKDGCITKEEMLDIMKSIYDMMGKYTYPALREEAPREHVESFFQKMDRNKDGVVTTIEEFIESCQK  
DEN  
IMRSMQLFDNVI

Fig. 10 Continued



## RAT 9QM DNA (CD:214-972)

CTCACTTGCTGCCCAAGGCTCCTGCTCCTGCCCCAGGACTCTGAGGTGGGCCCTAAAACCCAGCGCTCTCTAAAGAAAAG  
CCTTGCCAGCCCCCTACTCCCGCCCCCAACCCACAGCAGGTGCTGCGCCGCCAGGGGGCGCTGTGTGAGCGCCCTATTCT  
GGCCACCCGGCGCCCCCTCCACGGCCCAGGCGGGAGCGGGGCGCCGGGGGCCATGCGGGGCCAAGGCAGAAAGGAGAGT  
TTGTCCGAATCCCGAGATCTGGACGGCTCCTATGACCAGCTTACGGGCCACCCCTCCAGGGCCCAGTAAAAAGCCCTGAA  
GCAGCGTTTCTCAAGCTGCTGCCGTGCTGCGGGCCCCAAGCCCTGCCCTCAGTCAGTGAAAAACAGCGTAGAGGATGAGT  
TTGAATTATCCACGGTGTGTACCCGACCTGAGGGCCTGGAACAACCTCCAGGAACAGACCAAGTTCACACGCAGAGAGCTC  
CAGGTCTGTACCGAGGCTTCAAGAACGAATGCCCCAGTGGGATTGTCAACGAGGAGAACTTCAAGCAGATTTATTCTCA  
GTTCTTTTCCCCAAGGAGACTCCAGCAACTATGCTACTTTTCTCTTCAATGCCTTTGACACCAACCACGATGGCTCTGTCA  
GTTTTGAGGACTTTGTGGCTGGTTTGTGCGGTGATTTCTCGGGGGACCATAGATGATAGACTGAGCTGGGCTTTCAACTTA  
TATGACCTCAACAAGGACGGCTGTATCACAAGGAGGAAATGCTTGACATTATGAAGTCCATCTATGACATGATGGGCAA  
GTACACATAACCTGCCCTCCGGGAGGAGGCCCAAGAGAACACGTGGAGAGCTTCTTCCAGAAGATGGACAGGAACAAGG  
ACGGCGTGGTGACCATCGAGGAATTCATCGAGTCTTGTCAACAGGACGAGAACATCATGAGGTCCATGCAGCTCTTTGAT  
AATGTCATCTAGCTCCCCAGGAGAGGGGTTAGTGTGCTCCTAGGGTGACCAGGCTGTAGTCTTAGTCCAGACGAACCTAA  
CCCTCTCTCTCCAGGCTGTCTCATCTTACCTGTACCTTGGGGCTGTAGGGATTCAATATCCTGGGGCTTCAGTAGTC  
CAGATCCCTGAGCTAAGTCACAAAAGTAGGCAAGAGTAGGCAAGCTAAATCTGGGGGCTTCCCAACCCCGACAGCTCTC  
ACCCCTTCTCAACTGATACCTAGTGCTGAGGACACCCCTGGTGTAGGGACCAAGTGGTTCTCCACCTTCTAGTCCCACTC  
TAGAAAACCATTAGACAGAAGGTCTCCTGCTATGGTGTCTTCCCCATCCCTAATCTCTTAGATTTTCTCAAGACTCCC  
TTCTCAGAGAACACGCTCTGTCCATGTCCCAGCTGGCTTCTCAGCCTAGCCTTTGAGGGCCCTGTGGGGAGGCGGGGAC  
AAGAAAGCAGAAAAGTCTTGGCCCCGAGCCAGTGGTTAGGTCTTAGGAATTGGCTGGAGTGGAGGCCAGAAAGCCTGGGC  
AGATGATGAGAGCCCAGCTGGGCTGTCACTGCAGGTTCCGGGGCCTACAGCCCTGGGTGAGCAGAGTATGAGTTCCAGAG  
CTTTCCAGAAGGTCTTAGCAATGTCCAGAAATTCACCGTACACTTCTCAGTGTCTTAGGAGGGCCCCGGGATCCAGATG  
TCTGGTTCATCCCTGAATCCTCTCCCTCCTTCTTGCTCGTATGGTGGGAGTGGTGGCCAGGGGAAGATGAGTGGTGTCCC  
GGATGATGCTGTCAAGGTCCCACCTCCCCTCCGGCTGTTCTCATGACAGCTGTTTGGTTCCTCATGACCCCTATCTAGA  
TGTAGAGGCATGGAGTGAGTCAGGGATTTCCCGAAGTTGAGTTTACCACCTCCTCCTAGTGGCTGCCTTAGGGGAATGGG  
AAGAACCAGTGTGGGGGCACCCATTAGAATCTTTGCCCGGCTCCTCACAATGCCCTAGGGTCCCCTAGGGTACCCGCTC  
CCTCTGTTTAGTCTACCCAGAGATGCTCCTGAGCTCACCTAGAGGGTAGGGACGGTAGGCTCCAGGTCCAACCTCTCCAG  
GTCAGCACCCCTGCCATGCTGCTGCTCCTCATTAAACAAACCTGCTTGTCTCCTCCTGCGCCCCCTTCTCAGTCAGCCAGGGT  
CTGAGGGGAAGGGCCTCCCGTTTCCCCATCCGTCAGACATGGTTGACTGCTTTGCATTTTGGGCTCTTCTATCTATTTTG  
TAAATAAGACATCAGATCCAATAAAACACACGGCTATGCACAAAAA

## RAT 9QM PROTEIN

MRGQGRKESLSERDLGSDYDQLTGHPGPSKKALKQRFLKLLPCCGPQALPSVSENSVEDEFELSTVCHRPEGLEQLQE  
QTKFTRRELQVLYRGFKNECPSGIVNEENFKQIYSQFFPQGDSSNYATFLNAPDTHDGSVSFEDFVAGLSVILRGITD  
DRLSWAFNLYDLNKDGCITKEEMLDIMKSIYDMMGKYTYPALREEAPREHVESFFQKMDRNDGVTIEEFIESCQDEN  
IMRSMQLFDNVI

Fig. 11

HUMAN 9QS DNA (CD:207-869)

CTCACCTGCTGCCTAGTGTTCCCTCTCCTGCTCCAGGACCTCCGGGTAGACCTCAGACCCCGGGCCCATTTCCAGACTCA  
GCCTCAGCCCGGACTTCCCCAGCCCCGACAGCACAGTAGGCCGCCAGGGGGCGCCGTGTGAGCGCCCTATCCCGGCCACC  
CGGCGCCCCCTCCACGGCCCCGGGCGGGAGCGGGGCGCCGGGGGCCATGCGGGGCCAGGGCCGCAAGGAGAGTTTGTCCG  
ATTCCCGAGACCTGACGGCTCCTACGACCAGCTCACGGACAGCGTGGACGATGAATTTGAATTGTCCACCGTGTGTAC  
CGGCCTGAGGGTCTGGAGCAGCTGCAGGAGCAAACCAAATTCACGCGCAAGGAGTTGCAGGTCTGTACCGGGGCTTCAA  
GAACGAATGTCCCAGCGGAATTGTCAATGAGGAGAACTTCAAGCAGATTTACTCCCAGTTCTTTCTCAAGGAGACTCCA  
GCACCTATGCCACTTTTCTCTTCAATGCCTTTGACACCAACCATGATGGCTCGGTCAGTTTGTAGGACTTTGTGGCTGGT  
TTGTCCGTGATTCTTCGGGGAACTGTAGATGACAGGCTTAATTGGGCCTTCAACCTGTATGACCTTAACAAGGACGGCTG  
CATCACCAAGGAGGAAATGCTTGACATCATGAAGTCCATCTATGACATGATGGGCAAGTACACGTACCCTGCACTCCGGG  
AGGAGGCCCAAGGGAACACGTGGAGAGCTTCTTCCAGAAGATGGACAGAAACAAGGATGGTGTGGTGACCATTGAGGAA  
TTCATTGAGTCTTGTCAAAAGGATGAGAACATCATGAGGTCCATGCAGCTCTTTGACAATGTCATCTAGCCCCCAGGAGA  
GGGGGTCAGTGTTTCTTGGGGGACCATGCTCTAACCCCTAGTCCAGGCGGACCTCACCCCTTCTCTTCCCAGGTCTATCCT  
CATCCTACGCCCTCCCTGGGGGCTGGAGGGATCCAAGAGCTTGGGGATTTCAGTAGTCCAGATCTCTGGAGCTGAAGGGGCC  
AGAGAGTGGGCAGAGTGCATCTCGGGGGGTGTTCCCAACTCCCACCAGCTCTCACCCCTTCTCTGCTGACACCCAGTGT  
TGAGAGTGGCCCTCCTGTAGGAATTGAGCGGTTCCCCACCTCCTACCCCTACTCTAGAAACACACTAGAGCGATGTCTCCT  
GCTATGGTGCCTTCCCCCATCCCTGACCTCATAAACATTTCCCCTAAGACTCCCCCTCTCAGAGAGAATGCTCCATTCTTGG  
CACTGGCTGGCTTCTCAGACCAGCCATTGAGAGCCCTGTGGGAGGGGGACAAGAATGTATAGGGAGAAATCTTGGGCCTG  
AGTCAATGGATAGGTCTTAGGAGGTGGGTGGGGTTGAGAATAGAAGGGCCTGGACAGATTATGATTGCTCAGGCATACCA  
GGTTATAGCTCCAAGTTCCACAGGTCTGCTACCACAGGCCATCAAATATAAGTTTCCAGGCTTTGCAGAAGACCTTGTC  
TCCTTAGAAATGCCCCAGAAATTTTCCACACCCCTCCTCGGTATCCATGGAGAGCCTGGGGCCAGATATCTGGCTCATCTC  
TGGCATTGCTTCTCTCCTTCCCTTCTGTCATGTGTTGGTGGTGGTGTGGTGGGGGAATGTGGATGGGGGATGTCTGGC  
TGATGCCTGCCAAAATTTTCATCCCACCCCTCCTTGCTTATCGTCCCTGTTTTGAGGGCTATGACTTGAGTTTTTGTTTCCC  
ATGTTCTCTATAGACTTGGGACCTTCTGAACTTGGGGCCTATCACTCCCCACAGTGGATGCCCTAGAAGGGAGAGGGAA  
GGAGGGAGGCAGGCATAGC

Fig. 12

## MONKEY 9QS DNA (CD:133-795)

CCCACGCGTCCGCCCACGCGTCCGCGGACGCGTGGGGTGCACTAGGCCGCCAGGGGGCGCCGTGTGAGCGCCCTATCCCG  
GCCACCCGGCGCCCCCTCCACGGACCGGGCGGGAGCGGGGCGCCGGGGCCATGCGGGGCCAGGGCCCAAGGAGAGTT  
TGTCCGATTCCCGAGACCTGGACGGATCCTACGACCAGCTCACGGACAGCGTGGAGGATGAATTTGAATTGTCCACCGTG  
TGTCACCGGCCTGAGGGTCTGGAGCAGCTGCAGGAGCAAACCAATTACGCGCAAGGAGTTGCAGGTCTGTACCGGGG  
CTTCAAGAACGAATGTCCGAGCGGAATTGTCAATGAGGAGAACTTCAAGCAAATTTACTCCCAGTTCTTTCCCTCAAGGAG  
ACTCCAGCACCTATGCCACTTTTCTCTTCAATGCCCTTGACACCAACCATGATGGCTCGGTACGTTTGTGAGGACTTTGTG  
GCTGGTTTGTCCGTGATTCTTCGGGGAACGTGTAGATGACAGGCTTAATTGGGCCCTCAACTTGTATGACCTCAACAAGGA  
CGGCTGCATCACCAAGGAGGAAATGCTTGACATCATGAAGTCCATCTATGACATGATGGGCAAGTACACATACCTGCAC  
TCCGGGAGGAGGCCCAAGGGAACATGTGGAGAACCTCTTCCAGAAGATGGACAGAAACAAGGATGGCGTGGTGACCATT  
GAGGAATTCATTGAGTCTTGTCAAAGGATGAGAACATCATGAGGTCCATGCAGCTCTTTGACAATGTCATCTAGCCCCC  
AGGAGAGGGGGTCACTGTTTCTCGGGGGACCATGCTCTAACCTAGTCCAGGTGGACCTCACCTTCTCTTCCCAGGTC  
TATCCTTGTCTAGGCCTCCCTGGGGGCTGGAGGGATCCAAGAGCTTGGGGATTCACTAGTCCAGATCTCTGGAGCTGAA  
GGGGCCAGAGAGTGGGCAGAGTGCATCTTGGGGGGTGTTCCTCAACTCCCACCAGCTTTCACCCGCTTCTGCTGACACC  
CAGTGTGAGAGTCCCCCTCCTGTAGGAACGTAGTGGTTCCCCACCTCCTACCCCCACTCTAGAAACACACTAGACAGAT  
GTCTCGTGCTATGGTGCTTCCCCCATCCCTGACTTCATAAACATTTCCCCTAAACTCCCTTCTCAGAGAGAATGCTCCA  
TTCTTGGCACTGGCTGGCTTCTCAGACCAGCCTTTGAGAGCCCTGTGGGAGGGGGACAAGAATGTATAGGGGAGAAATCT  
TGGGCCTGAGTCAATGGATAGGTCTTAGGAGGTGGCTGGGGTTGAGAATAGAAAGGCCTGGACACAATGTGATTGCTCAG  
GCATACCAAGTTATAGCTCCAAGTTCCACAGGTCTGCTACCACAGGCCATCAAAATATAAGTTTCCAGGCTTTGCAGAAG  
ACCTTGTCTCCTTGGAAATGCCCCAGATATTTCCATACCCCTCCTCGATATCCATGGAGAGCCTGGGGCTAGATATCTGG  
CATATCCCTGGCATTTGCTTCTCTCCTTCCCTTCTGCTGCTGTTGGTGGTGGTGTGGCAGGGGAATGTGGATAGGAGAT  
GTCTTGGCAGATGCCTGCCAAAGTTTCATCCCACCCTCCCTGCTCATCGCCCCCTGTTTGTAGGGCTGTGACTTGAGTTTT  
TGTTTCCCATGTTCTCTATAGACTTGGGACCTTCTGAACTTGGGGCCTATCACTCCCCACAGTGGATGCCTTAGAAGGG  
AGAGGGAAGGAGGGAGGCAGGCATAGCATCTGAACCCAGTGTGGGGGCATTCACTAGGATCTTCAATCAACCCGGGCTCT  
CCCCAACCCCCAGATAACCTCCTCAGTTCCCTAGAGTCTCCTCTTGCTCTACTCAATCTACCCAGAGATGCCCCCTAGC  
ACACTCAGAGGGCAGGGACCATAGGACCCAGGTTCCAACCCCATTTGTCAGCAGCCAGCCATGCTGCCATCCCTTAGCAC  
ACCTGCTCGTCCCATTCAGCTTACCTCCCAGTCAGCCAGAATCTGAGGGGAGGGCCCCCAGAGAGCCCCCTTCCCCATC  
AGAAGACTGTTGACTGCTTTGCATTTTGGGCTCTTCTATATATTTGTAAAAATAAGAACTATACCAGATCTAATAAAACA  
CAATGGCTATGCAAAAAAAAAAAAAAAAAAAAAA

## MONKEY 9QS PROTEIN

MRGQGRKESLSDSRDLDGSYDQLTDSVEDEFELSTVCHRPEGLEQLQEQTKEFTRKELQVLYRGFKNECPSGIVNEENFKQ  
IYSQFFPQGDSSSTYATFLFNAFDTNHDGSVSFEDFVAGLSVILRGTVDDRLNWFNLYDLNKDGCITKEEMLDIMKSIYD  
MMGKYTYPALREEAPREHVENFFQKMDRNKDGVVTFIEEFIESQKDENIMRSMQLFDNVI

Fig. 13

RAT 9QC DNA (CD:208-966)

TGCTGCCCAAGGCTCCTGCTCCTGCCCCAGGACTCTGAGGTGGGCCCTAAAACCCAGCGCTCTCTAAAGAAAAGCCTTGC  
CAGCCCCTACTCCCGCCCCCAACCCAGCAGGTCGCTGCGCCGCCAGGGGGCGCTGTGTGAGCGCCCTATTCTGGCCAC  
CCGGCGCCCCCTCCACGGCCAGGCGGGAGCGGGGGCGCCGGGGGCCATGCGGGGCCAAGGCAGAAAGGAGAGTTTGTCC  
GAATCCCGAGATCTGGACGGCTCCTATGACCAGCTTACGGGCCACCCTCCAGGGCCCAGTAAAAAGCCCTGAAGCAGCG  
TTTCCTCAAGCTGCTGCCGTGCTGCGGGCCCCAAGCCCTGCCCTCAGTCAGTGAAAACAGCGTAgAGGATGAGTTTGAAT  
TATCCACGGTGTGTACCGACCTGAGGGCCTGGAACAACCTCCAGGAACAGACCAAGTTCACACGCAGAGAGCTGCAGGTC  
CTGTACCGAGGCTTCAAGAACGAATGCCCCAGTGGGATTGTCAACGAGGAGAACTTCAAGCAGATTATTCTCAGTTCTT  
TCCCCAAGGAGACTCCAGCAACTATGCTACTTTTCTCTTCAATGCCCTTTGACACCAACCACGATGGCTCTGTCAGTTTGTG  
AGGACTTTGTGGCTGGTTTGTCTGGTGATTCTTCGGGGGACCATAGATGATAGACTGAGCTGGGCTTCAACTTATATGAC  
CTCAACAAGGACGGCTGTATCACAAGGAGGAAATGCTTGACATTATGAAGTCCATCTATGACATGATGGGCAAGTACAC  
ATACCCTGCCCTCCGGGAGGAGGCCCAAGAGAACACGTGGAGAGCTTCTCCAGAAGATGGACAGGAACAAGGACGGCG  
TGGTGACCATCGAGGAATTCATCGAGTCTTGTC AACAGGACGAGAACATCATGAGGTCCATGCAGCTCTCACCCCTTCTC  
AACTGATACCTAGTGCTGAGGACACCCCTGGTGTAGGGACCAAGTGGTTCTCCACCTTCTAGTCCCACTCTAGAAACCAC  
ATTAGACAGAAGGTCTCCTGCTATGGTGCTTTCCCCATCCCTAATCTCTTAGATTTTCTCAAGACTCCCTTCTCAGAGA  
ACACGCTCTGTCCATGTCCCAGCTGGCTTCTCAGCCTAGCCCTTTGAGGGCCCTGTGGGGAGGCGGGGACAAGAAAGCAG  
AAAAGTCTTGGCCCCGAGCCAGTGGTTAGGTCTTAGGAATTGGCTGGAGTGGAGGCCAGAAAGCCTGGGCAGATGATGAG  
AGCCCAGCTGGGCTGTCACTGCAGGTTCGGGGCCCTACAGCCCTGGGTGAGCAGAGTATGAGTTCCAGACTTTCCAGAA  
GGTCTTAGCAATGTCCCAGAAATTCACCGTACACTTCTCAGTGTCTTAGGAGGGCCCCGGGATCCAGATGTCTGGTTTCT  
CCCTGAATCTCTCCCTCTTCTTGCTCGTATGGTGGGAGTGGTGGCCAGGGGAAGATGAGTGGTGTCCCGGATGATGCC  
TGTCAAGGTCCCACCTCCCCTCCGGCTGTCTCATGACAGCTGTTTGGTTCTCCATGACCCCTATCTAGATGTAGAGGCA  
TGGAGTGAGTCAGGGATTTCCCGAACTTGAGTTTACCACTCCTCCTAGTGGCTGCCCTTAGGGGAATGGGAAGAACCAG  
TGTGGGGGCACCCATTAGAATCTTTGCCCGGCTCCTCACAATGCCCTAGGGTCCCCTAGGGTACCCGCTCCCTCTGTTTA  
GTCTACCCAGAGATGCTCCTGAGCTCACCTAGAGGGTAGGGACGGTAGGCTCCAGGTCCAACCTCTCCAGGTCAGCACCC  
TGCCATGCTGCTGCTCCTCATTAACAAACCTGCTTGTCTCCTCCTGCGCCCCCTTCTCAGTCAGCCAGGTCTGAGGGGAA  
GGGCTCCCGTTTCCCCATCCGTGAGCATGGTTGACTGCTTTGCATTTTGGGCTCTTCTATCTATTTTGTAAAATAAGA  
CATCAGATCCAATAAAACACACGGCTATGCACAAAAAAAAAAAAAAAAAAAAAAAAA

RAT 9QC PROTEIN

MRGQGRKESLSERDLDSYDQLTGHPGPSKKALKQRFLKLLPCCGPQALPSVSENSVEDEFELSTVCHRPEGLEQLQE  
QTKFTRRELQVLYRGFKNECPSGIVNEENFKQIYSQFFPQGDSSNYATFLFNAFDTNHDGSVSFEDFVAGLSVILRGITD  
DRLSWAFNLYDLNKDGCITKEEMLDIMKSIYDMMGKYTYPALREEAPREHVESFFQKMDRNKDGVVTTIEEFIESCQDEN  
IMRSMQLSPLLN

Fig. 14

RAT 8T (9Q SPLICE VARIANT) DNA (MAY NOT BE FULL LENGTH, CD: 1-678)

ATGAACCACTGCCCTCGCAGGTGCCGGAGCCCGTTGGGGCAGGCAGCTCGATCTCTCTACCAGTTGGTAACTGGGTCGCT  
GTGCCCAGACAGCGTAGAGGATGAGTTTGAATTATCCACGGTGTGTACCCGACCTGAGGGCCTGGAACAACCTCCAGGAAC  
AGACCAAGTTCACACGCAGAGAGCTGCAGGTCTGTACCGAGGCTTCAAGAACGAATGCCCCAGTGGGATTGTCAACGAG  
GAGAACTTCAAGCAGATTTATTCTCAGTTCTTTCCCCAAGGAGACTCCAGCAACTATGCTACTTTTCTCTTCAATGCCCTT  
TGACACCAACCACGATGGCTCTGTGTCAGTTTGTGAGGACTTTGTGGCTGGTTTGTCCGTGATTCTTCGGGGGACCATAGATG  
ATAGACTGAGCTGGGCTTTCAACTTATATGACCTCAACAAGGACGGCTGTATCACAAGGAGGAAATGCTTGACATTATG  
AAGTCCATCTATGACATGATGGGCAAGTACACATACCCTGCCCTCCGGGAGGAGGCCCCAAGAGAACACGTGGAGAGCTT  
CTTCCAGAAGATGGACAGGAACAAGGACGGCGTGGTGACCATCGAGGAATTCATCGAGTCTTGTCAACAGGACGAGAACA  
TCATGAGGTCCATGCAGCTCTTTGATAATGTATCTAGCTCCCCAGGGAGAGGGGTAGTGTGTCTAGGGTGACCAGGC  
TGTAGTCCTAGTCCAGACGAACCTAACCCCTCTCTCTCCAGGCCTGTCTCATCTTACCTGTACCCTGGGGGCTGTAGGGA  
TTCAATATCCTGGGGCTTCAGTAGTCCAGATCCCTGAGCTAAGTCACAAAAGTAGGCAAGAGTAGGCAAGCTAAATCTGG  
GGGCTTCCCAACCCCGACAGCTCTCACCCCTTCTCAACTGATACCTAGTGTGAGGACACCCCTGGTGTAGGGACCAAG  
TGGTTCTCCACCTTCTAGTCCCACTCTAGAAACCACATTAGACAGAAGGTCTCTGCTATGGTGCTTTCCCCATCCCTAA  
TCTCTTAGATTTTCTCAAGACTCCCTTCTCAGAGAACACGCTCTGTCCATGTCCCCAGCTGGCTTCTCAGCCTAGCCTT  
TGAGGGCCCTGTGGGGAGGCGGGGACAAGAAAGCAGAAAAGTCTTGGCCCCGAGCTAGTGGTTAGGTCTTAGGAATTGGC  
TGGAGTGGAGGCCAGAAAGCCTGGGCAGATGATGAGAGCCAGCTGGGCTGTCACTGCAGGTTCCAGGGCCTACAGCCCT  
GGGTCAGCAGAGTATGAGTTCCAGACTTTCCAGAAGTCTTAGCAATGTCCAGAAATTCACCATACACTTCTCAGTG  
TCCCGGATGATGCCTGTCAAGGTCCCACCTCCCTCCGGCTGTTCTCATGACAGCTGTTTGGTTCTCCATGACCCCTATC  
TAGATGTAGAGGCATGGAGTGAGTCAGGGATTTCCCGAACTTGAGTTTACCCTCCTCCTAGTGGCTGCCTTAGGGGAA  
TGGGAAGAACCAGTGTGGGGGCACCCATTAGAATCTTTGCCCGGTTCTCACAATGCCCTAGGGTCCCTAGGGTACCC  
GCTCCCTCTGTTTAGTCTACCCAGAGATGCTCCTGAGCTCACCTAGAGGGTAGGGACGGTAGGCTCCAGGTCCAACCTCT  
CCAGGTGAGCACCTGCCATGCTGCTGCTCCTCATTAAACAACCTGCTTGTCTCCTCCTGCGCCCCCTTCTCAGTCAGCCA  
GGGTCTGAGGGGAAGGCCTCCCGTTTCCCCATCCGTGAGACATGGTTGACTGCTTTGCATTTTGGGCTCTTCTATCTAT  
TTTGTAATAATAAGACATCAGATCCAATAAAACACACGGCTATGCACAAAAAAAAAAAAAAAAAAAAA

RAT 8T (9Q SPLICE VARIANT) PROTEIN (MAY NOT BE FULL LENGTH)

MNHCPRRCRSPLGQAARSLYQLVTGSLSPDSVEDEFELSTVCHRPEGLQLQEQTKFTRRELQVLYRGFKNECPSGIVNE  
ENFKQIYSQFFPQGDSSNYATFLFNAFDTNHDGSVSFEDFVAGLSVILRGTTIDRLSWAFNLYDLNKDGCITKEMLDIM  
KSIYDMMGKYTYPALREEAPREHVESFFQKMDRNKDGVTITIEEFIESCQQDENIMRSMQLFDNVI

Fig. 15

>human KChIP3 cds=1-7:

ATGCAGCCGGCTAAGGAAGTGACAAAGGCGTCGGACGGCAGCCTCCTGGGGGACCTCGGGC  
ACACACCACTTAGCAAGAA  
GGAGGGTATCAAGTGGCAGAGGCCGAGGCTCAGCCGCCAGGCTTTGATGAGATGCTGCCTG  
GTCAAGTGGATCCTGTCCA  
GCACAGCCCCACAGGGCTCAGATAGCAGCGACAGTGAGCTGGAGCTGTCCACGGTGCGCCA  
CCAGCCAGAGGGGCTGGAC  
CAGCTGCAGGCCAGACCAAGTTCACCAAGAAGGAGCTGCAGTCTCTCTACAGGGGCTTTA  
AGAATGAGTGTCCCACGGG  
CCTGGTGGACGAAGACACCTTCAAACCTCATTTACGCGCAGTTCTTCCCTCAGGGAGATGCCA  
CCACCTATGCACACTTCC  
TCTTCAACGCCCTTTGATGCGGACGGGAACGGGGCCATCCACTTTGAGGACTTTGTGGTTGGC  
CTCTCCATCCTGCTGCGG  
GGCACAGTCCACGAGAAGCTCAAGTGGGCCTTTAATCTCTACGACATTAACAAGGATGGCT  
ACATCACCAAAGAGGAGAT  
GCTGGCCATCATGAAGTCCATCTATGACATGATGGGCCGCCACACCTACCCCATCCTGCGGG  
AGGACGCGCCGGCGGAGC  
ACGTGGAGAGGTTCTTCGAGAAAATGGACCGGAACCAGGATGGGGTAGTGACCATTGAAGA  
GTTCTTGGAGGCCTGTCTAG  
AAGGATGAGAACATCATGAGCTCCATGCAGCTGTTTGAGAATGTCATCTAGgacacgtccaaaggagt  
gcatggccacag  
ccacctccaccccccaagaaacctccatcctgcccaggagcagcctccaagaaacttttaaaaaatagatttgcaaaaagtg  
aacagattgctacagccattcatctgggctggcagaggggac  
agagttcagggaggggctgagctctggctaggggcccaggtccaggagccccagccagccctcccaggccagcgagggcgag  
gctgcctctgggtgagtggtgacagagcaggtctgcaggccaccagctgctggatgtcaccaagaaggggctcgagtgc  
ccctgcaggggaggggtccaatctccgggtgtgagcccactcgtcccgttctccattctgctttcttgccacacagtgggc  
cgccccaggtctcccctggtctcctccccgtagccactctctgcccactacctatgcttctagaagcccctcacctcag  
gaccccagagggaccagctggggggcaggggggagagggggtaatggaggccaagcctgcagcttctggaaattcttcc  
ctgggggtcccaggatcccctgctactccactgacctggaagagctgggtaccaggccaccactgtggggcaagcctga  
gtggtgaggggcccactggggcccattctccctccatggcaggaaggcggggatttcaagtttagggattgggtcggtgt  
ggagaatctgagggcactctctgcccagctccacaggggtgggatgagcctctccttgccccagtcctgggtcagtggaat  
gcagtggtggggctgtacacaccctccagcacagactgttccctccaaggtcctcttaggtcccgggaggaacgtggtt  
cagagactggcagccagggagccccggggcagagctcagaggagtctgggaaggggctgtccctcctcttctgtagtgc  
ccctcccatggcccagcagcttaggtgagccccctctcctgaagcagtgctgcgctccctctgcttgcaaaaaagcac  
aagcattccttagcagctcaggcgcagccctagtgaggagccagcacactgcttctcggaggccaggccctcctgctggc  
tgaggcttggggccagtagccccaatatggtggccctggggaagaggcccttgggggtctgctctgtgctgggatcagtg  
gggccccaaagcccagcccgggtgaccaacattcaaaagcacaaaaccctggggactctgcttgggtgtcccctccatctg  
gggatggagaatgccagccaaagctggagccaatggtgagggctgagagggctgtggctgggtggtcagcagaaccccc  
caggaggagagagatgctgctcccgctgattggggcctcaccagaaggaaccgggtcccaggccgcatggccctcca  
ggaacattcccacataatacattccatcacagccagcccagctccactcagggctggcccggggagtccccgtgtgccc  
aagaggctagccccaggggtgagcagggccctcagaggaaaggcagtatggcggaggccatgggggcccctcggcattcac  
acacagcctggcctcccctgaggagctgcatggagcctgggtccagggtccagggtgactgggggctctgctccagg  
agggcatcagcttcccctgggtcagggatcttctccctcccctcaccgctgccagccctcccagctggtgtoactctg  
cctctaaggccaaggcctcaggagagcatcaccaccacaccccctgccggccttggccttggggccagactgggtgcacag  
cccaaccaggaggggtctgctcccacgctgggacacagaccggccgcatgtctgcatggcagaagcgtctcccaggcc  
acggcctgggaggggtggttctgttctcagcatccactaatattcagtcctgtatatattttaataaaaataaacttgacaaa  
ggaaaaaaaaaaaaaaaaaattcctgcccgcgcttctcca

Fig.16

>human KChIP3  
MQPAKEVTKASDGSLLGDLGHTPLSKKEGIKWQRPRLSRQALMRCCLVKWILSSTAPQGSDDSSD  
SELELSTVRHQPEGLD  
QLQAQTKFTKKELQSLYRGFKNECPTGLVDEDTFKLIYAQFFPQGDATTYAHFLFNAFDADGNG  
AIHFEDFVVGLSILLR  
GTVHEKLRWAFNLYDINKDGYITKEEMLAIMKSIYDMMGRHTYPILREDAPAEHVERFFEKMD  
RNQDGVVTIEEFLEACQ  
KDENIMSSMQLFENVI

Fig.16 Continued

RAT P19 DNA (FIRST PASS, PARTIAL; CD:1-330)

TTTGAGGACTTTGTGGTTGGGCTCTCCATCCTGCTTCGAGGGACCGTCCATGAGAAGCTCAAGTGGGCCTTCAATCTCTA  
CGACATCAACAAGGACGGTTACATCACCAAAGAGGAGATGCTGGCCATCATGAAGTCCATCTACGACATGATGGGCCGCC  
ACACCTACCCTATCCTGCGGGAGGACGCACCTCTGGAGCATGTGGAGAGGTTCTTCCAGAAAATGGACAGGAACCAGGAT  
GGAGTAGTGACTATTGATGAATTTCTGGAGACTTGTTCAGAAGGACGAGAACATCATGAGCTCCATGCAGCTGTTTGAGAA  
CGTCATCTAGGACATGTAGGAGGGGACCCCTGGGTGGCCATGGGTTCCTCAACCCAGAGAAGCCTCAATCCTGACAGGAGAA  
GCCTCTATGAGAAACATTTTCTAATATATTTGCAAAAAGTG

RAT P19 PROTEIN (PARTIAL)

FEDFVVGLSILLRGTVHEKWKWAFNLYDINKDGYITKEEMLAIMKSIYDMMGRHTYPILREDAPLEHVERFFQKMDRNQD  
GVVTIDEFLETQKQDENIMSSMQLFENVI

Fig.17



MOUSE P19 DNA (CD: 49-819)

CGGGCTGCAAAGCGGGAAGSTTAGTGACGGTCCCTTTTCAGCAGCAGAGATGCAGAGGACCAAGGAAGCCGTGAAGGCATC  
AGATGGCAACCTCCTGGGAGATCCTGGGCGCATACCACTGAGCAAGAGGGAAAGCATCAAGTGGCAAAGGCCACGGTTCA  
CCCCGCCAGGCCCTGATGCGTTGCTGCTTAATCAAGTGGATCCTGTCCAGTGCTGCCCCACAAGGCTCAGACAGCAGTGAC  
AGTGAACCTGGAGTTATCCACGGTGCGCCATCAGCCAGAGGGCTTGGACCAGCTACAAGCTCAGACCAAGTTCCACCAAGAA  
GGAGCTGCAGTCCCTTTACCGAGGCTTCAAGAATGAGTGTCCACAGGCCTGGTGGATGAAGACACCTTCAAACTCATTT  
ATTCCCAGTTCTTCCCTCAGGGAGATGCCACCACCTATGCACACTTCTCTCAATGCCTTTGATGCTGATGGGAACGGG  
GCCATCCACTTTGAGGACTTTGTGGTTGGGCTCTCCATCCTGCTTCGAGGGACGGTCCATGAGAAGCTCAAGTGGGCCTT  
CAATCTCTATGACATTAACAAGGATGGTTGCATCACCAAGGAGGAGATGCTGGCCATCATGAAGTCCATCTACGACATGA  
TGGGCCGCCACACCTACCCCATCCTGCGGGAGGATGCACCCCTGGAGCATGTGGAGAGGTTCTTTCAGAAAATGGACAGG  
AACCAGGATGGAGTGGTGACCATTTGATGTATTTCTGGAGACTTGTCAAGAAGGATGAGAACATCATGAACCTCATGCAGCT  
GTTTGAGAACGTCATCTAGGACATGTGGGAGGGGACCCAGTGGTCAATTGCTTCTCAACCCAGAGSAGCCTCAATCCTGA  
CAGGAGAAGCCTCTATGAGAAACATTTTCTAATATATTTGCAAAAAGTGAGCAGTTTACTTCCAAGACACAGCCACCGT  
CACACACAGACACAGACATACAGACACACACACACACACATGGTTCTCTGGCTTGGCCAAGGAAGTGGCAGCC  
AGAAGGCACCCCCGCTATTTCCTAGGTCAATAAAAAAGGCTGCCTCTGGGATGGCCAGCCCTGGCTAGATGTTACCCACA  
AGGAACTCAGAGATCGAGAGGACCAGGTCTACAAAGCTAAGGTCCCTGTGTCTTTTCTACCACTCGGGAGATCAAACCTAC  
TCCCTGCCTATGGACCCATGCTCTTAGGAAGCTCCCAGAACTCCAAGGGGACAAAGAGGGGAGAGGTCTATAGGAAGAA  
ATGGTTTTTGAAGCTGGGCTTGCAGCCTTATGCTAATGATCACCTGGGGTCTTGAACCCGAGTGCCAGGCTACCTACTA  
TGCCGTGAGCTTAGATAGTGAGGGGGCATTGGACTAAGACCTCCTGTAAGAGTGGGGCAGGATTGAGGTTTTTGGAGAAA  
CTGAGGAAACAATTTGTCCATACCACTGGGTGAAGACTGCTGGCCAGTGGGAATGTGGCTGGTGGAGATTTCCCAACTTC  
CAGCACCAGGATGGCCTCTCCAAGGTCTCTTTGATTCCCTGGGGAGATCACCTGGCTCATAGACTGACAACCAGGGAAC  
TGGGCTGAAATGGGAGGTCTGGTAGGGGGCATCCCCCTCCTTTTCCCTGGCCACTTGCCACCCAGTTTCTTAACACAGTG  
GATCGGCCACACCTCTGTGGCTGCCCTTGAACAGACTCATCCCGACCAAGACAAAAAGCACTAACTCCTAGCAGCTCAG  
GCCAAGCCCACAAGGGAAGGCCTGGGTCCCTGCAGCCCTGATTCACTGGCCGAGGAAGACGCTCAGACATCCATCCTGTA  
CCTCGGAGCCTTGGGGGTCTCACAGCCCTTTCCAGCCAGCTCGCCAACATTCTAAAGCACAAACCTGCGGATTCTGCT  
TGCTTGGGCTGCGCCCTGGGGATTGAAGGCCACTGTTAACCCTAAGCTGGAGCTAGCCCTGAGGGCTGGGGACCTGTGAC  
CAGGCAACAGGTGAGCAGACCCCTCAGGAGGAGAGAGAGCTGTTTCTGCCTCCCCAGGCCTCGCCCAGAAGGAACAGTGT  
CCAAGAAGCATGTTTCTGGAGGAACATCCCCACAAAAGTACATTCCATCATCTGAAGCCCCGGTCTCTGCTCAGGCCTGC  
CTCTGAAAGTCCACGTGTGTTCCCCAGAAGGCCAGCCCCAAGATAAGGGAGGTCTTAGAGGAAGGACAGGGTGACAACA  
CCCCATACACAGGTGGACCCCCCTCTGAGGACTGTACTGACCCCATCTCCATCCTGACCGGGGCCCTTCTTTTACCCGA  
TCTACAGACCACAGTTCTCCCTGGCTCAGGGACCCCCGTGCCCCAGTCTGACTCTTCCCATCGAGGTCCCTGTCTTGT  
GAAAGCCAAGGCCACGGGAAAAGGCCACCACTCTAACCTGCTGCATCCCTTAGCCTCTGGCTGCACGCCCAACCTGGAG  
GGGTCTGTCCCTTTGCAGGGACACAGACTGGCCGCATGTCCGCATGGCAGAAGCGTCTCCCTTGGGTGCAGCCTGGAAG  
GGTGGTTTCTGTCTCAGCGCCCAACATATTCAGTCTATATATTTAATAAAAGAACTTGACAAAGGAAAAA  
AAAA

Fig. 18

>AI 352454 (partial) cds = 1-339

CACGAGGTGGAAAGCATTTTCGGCTCAGCTGGAGGAGGCCAGCTCTACAGGCGGTTTCCTGT  
ACGCTCAGAACAGCACCAA  
GCGCAGCATTAAAGAGCGGCTCATGAAGCTCTTGCCCTGCTCAGCTGCCAAAACGTCGTCTC  
CTGCTATTCAAAACAGCG  
TGGAAGATGAACTGGAGATGGCCACCGTCAGGCATCGGCCCGAAGCCCTTGAGCTTCTGGA  
AGCCCAGAGCAAATTTACC  
AAGAAAGAGCTTCAGATCCTTTACAGAGGATTTAAGAACGTAAGAACTTCTTTTTGACTTT  
ACCTTCACACAATTGCCA  
GAGGAGCATTGAGAAATGAgaggaaaagggggaaaatatccattctatgagaagcccatcatatgtatatttcatact  
gaccttcccagataggaatataatcagtatctgtggactttgaatctctgtggcacacccatgctggcatactgtaatt  
gcccattaacaananagtttttgagaaaaaaaaaaaaaaaaaaaaaaaaaaaaa

>AI352454

HEVESISAQLEASSTGGFLYAQNSTKRSIKERLMKLLPCSAAKTSSPAIQNSVEDELEMATVRHR  
PEALELLEAQSKFT  
KKELQILYRGFKNVRTFFLTLPSEHSQRSIEK

Fig. 19

P193 (AA349365) DNA (CD:2-127,patial)

TGAAAGGTTCTTCGAGAAAATGCACCGGAACCAGGATGGGGTAGTGACCATTGAAGAGTTCCTGGAGG  
CTGTCAGAAAGGATGAGAACATCATGAGCTCCATGCAGCTGTTTGAGAATGTCATCTAGGACACGTCCAAA  
GGAGTGTCATGGCCACAGCCACCTCCACCCCCAAGAAACCTCCATCCTGCCAGGAGCAGCCTCCAAGAAA  
CTTTTAAAAAATAGATTTGCAAAAAGTGAACAGATTGCTACACACACACACACACACACACACACAC  
ACACACACACAGCCATTTCATCTGGGCTGGCAGAGGGGACAGAGTTCAGGGAGGGGCTGAGTCTGGCTAG  
GGGCCGAGTCCAGGAGCCCCAGCCAGCCCTTCCCAGGCCAGCGAGGCGAGGCTGCCTCTGGGTGAGTGG  
CTGACAGAGCAGGTCTGCAGGCCACCAGCTGCTGGATGTCACCAAGAAGGGGCTCGAGTGCCCCCTGCAG  
GGGAGGGTCCAATCTCCGGTGTGAGCCACCTCGTCCCGTTCTCCATTCTGCTTTCTTGCCACACAGTGGG  
CCGGCCCCCAGGCTCCCCCTGGTCTCCTCCCCGTAGCCACTCTCTGCCCACTACCTATGCTTCTAGAAAGCCC  
CTCACCTCAGGACCCCAGAGGACCAGCTGGGGGGCAGGGGGGAGAGGGGGTAATGGAGGCCAAGCCT  
GCAGCTTTCTGGAAATTTCTTCCCTGGGGGTCCAGGATCCCCCTGCTACTCCACTNACCTGGAAGAGCTGG  
GTACCAGGCCACCCACTGTGGGGCAAGCCTGAGTGGTGGAGGGGCCACTGGGCCCCATTCTCCCTCCATGG  
CAGGAAGGCGGGGATTTCAAGTTTAGGGATTGGGTCTGGTGGGAGAATCTGAGGGCACTCTCTGCCAG  
CTCCACAGGGTGGGATGAGCCTCTCCTTGCCCCAGTCCCTGGTTTCAGTGGGAATGCAGTGGGTGGGGCIGT  
ACACACCCCTCCAGCACAGACTGTTCCCTCCAAGGTCTCTTAGGTCCCGGGAGGAACGTGGTTCAGAGAC  
TGGCAGCCAGGGAGCCCCGGGGCAGAGCTCAGAGGAGTCTGGGAAGGGGCGTGTCCCTCTCTTCTGTA  
GTGCCCTCCCCATGGCCCAGCAGCTTGGCTGAGCCCCCTCTCCTGAAGCAGTGTGCGCGTCCCTCTGCCTT  
GCACAAAAGCACAAAGCATTCCTTAGCAGCTCAGGCGCAGCCCTAGTGGGAGCCCAGCACACTGCTTCT  
CGGAGGCCAGGCCCTCTGCTGGCTGAGGCTTGGGCCAGTAGCCCCAATATGGTGGCCCTGGGGAAGA  
GGCCTTGGGGGTCTGCTCTGTGCTTGGGATCAGTGGGGCCCCAAAGCCCAGCCCGGCTGACCAACATTCA  
AAAGCACAAACCCCTGGGGACTCTGCTTGGCTGTCCCCCTCCATCTGGGGATGGAGAATGCCAGCCCAAAG  
CTGGAGCCAATGGTGGGGCTGAGAGGGCTGTGGCTGGGTGGTTCAGCAGAAACCCCCAGGAGGAGAGA  
GATGCTGCTCCCGCCTGATTTGGGGCTCACCACAGAAAGGAACCCGGTCCCAGGCCGATGGCCCCCTCAGG  
AACATTCCACATAATACATTCCATCACAGCCAGCCCAGCTCCACTCAGGGCTGGCCCCGGGAGTCCCCG  
TGTGCCCAAGAGGCTAGCCCCAGGGTGGCAGGGCCCTCAGAGGAAAGGCAGTATGGCGGAGGCCATG  
GGGGCCCCCTCGGCATTACACACAGCCTGGCCCTCCCCCTGCGGAGCTGCATGGACGCTGGCTCCAGGCTC  
CAGGCTGACTGGGGGCTCTGCCTCCAGGAGGGCATCAGCTTTCCCTGGCTCAGGGATCTTCTCCCTCCC  
CTCACCCGCTGCCAGCCCTCCAGCTGGTGTCACTCTGCCTCTAAGGCCAAGGCCCTCAGGAGAGCATCA  
CCACCACACCCCTGCCGGCCTTGGCCTTGGGGCCAGACTGGCTGCACAGCCCAACCAGGAGGGGTCTGC  
CTCCACGCTGGGACACAGACCGGCCGATGTCTGCATGGCAGAAGCGTCTCCCTTGGCCACGGCCTGGG  
AGGGTGGTTCTCTTCTCAGCATCCACTAATATTAGTCTGTATATTTTAATAAAATAAACTTGACAAAG  
GAAAAAAAAAAAAAAAAA

P193 PROTEIN (PARTIAL)

ERFFEKMDRNQDGVVTIEEFLEACQKDENIMSSMQLFENV

Fig. 20

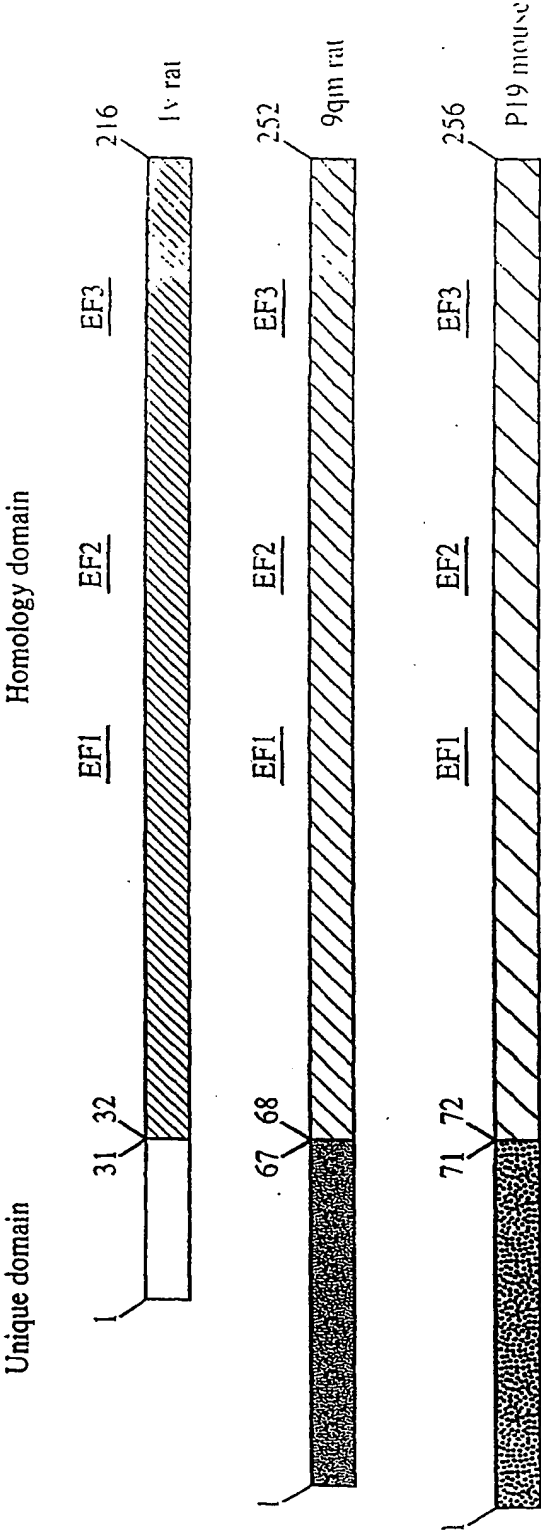


Fig. 21

Human 9q genomic DNA sequences:

A. exon1 sequence (with introns included):

CGGGAGGAGAGAGGCAGCTCGGCTCGGCTCCGCGCTCAGCTCCGCTCTGCCTCCGGCTCTGCGCTCACCTGCTGCCT  
AGTGTTCCTCTCCTGCTCCAGGACCTCCGGGTAGACCTCAGACCCCGGGCCCATTCAGACTCAGCCTCAGCCCG  
GACTTCCCCAGCCCCGACAGCACAGTAGGCCGCCAGGGGGCGCGTGTGAGCGCCCTATCCCGGCCACCCGGCGCCC  
CCTCCACGGCCCCGGGCGGAGCGGGGCGCCGGGGGCCATGCGGGGCCAGGGCCGCAAGGAGAGTTTGTCCGATTCC  
CGAGACCTGGACGGCTCTCAGACAGCTCACGGGTGAGTCACTGACGTGGGGGTGCGGGGAGGAGGGTGGATTCC  
ATTCCCTCCAGACCTTCCGCTCTCCGACCCCGGCTTGGCCCGCACCAACACTCTGCCCCATTCCAGGCATCTTA  
TGGCCGGTCTGGGCGGAGGACACTGGGGTTCAAAGCCTTGGGTCCCGCAGGGGTGGGGAGGAACAGAAGAGGCA  
GGTGTGGAGAGGCAGCAGGTGTGGGCGTATGTGACACAGGGCTGAGAGGGTGTCTGGAGTGGGAGGTGTTACCGTGC  
GTGAGCAGCTGTCTTCTGTGTGTGTGTGTGTGTGTGCGCGCGCACCTCCACAGCTGGTGGCATGTGCCCTGGGC  
TTGGTGACAGCTAGGGT  
GGACCTGGT  
GTATGAAAGTGGT

B. Exon 2-11 sequence (with introns included):

AGCCNANTGGGTGNCATGTGTATGCATCCTGTTACTTAGGTACATTTGTATATGTTGTGTAAAGGAGTACCAGGT  
CAATGT  
ATCACTGT  
ACCCAGGCAATCCTTGGGTGTGACATCATCCTGTGAGAGGTCCAGCCATGGCACTTGAGCCAAGGGTACTAGGTACAGCA  
AAGACATTGAGGCCACTGCCACCTCATCCTTGGCGCCTCGCTGTACCGGCCACGTCCCATTAACCAAGGTATGTA  
GCTTCACTCTATGGACTCAGTGGGCTCCCTTAACCCGATTCGACACCCCTTGCATTCTCTTCTCCCTTAAAT  
CCTCCCCAGCCCGTCCCATGATGGGGTTGATTTGTGACTGGCGGGAGGGGACAGGGAACAGAGGGACCCCGGA  
GTTAATGTGCTTCTTGGGGTCTTCTCTCTCTCNCAGGCCACCTCCAGGGCCACTAAAAAGCGCTGAAGCAGCGA  
TTCTCAAGCTGTGCGGAGCTGCGGGCCCAAGCCCTGCCCTCAGTCAGTGAAAGCAAGTGCTCTCATGTGCTTC  
CCGGGCGGGGCTCGATGTGTGCGT  
CATGTTCCAGGCTTGATGT  
GCGT  
TGATGT  
GT  
TGGCGAGGGCGGGT  
TGT  
ACCATTTGTGACAGGNTGAGGCCTGAGCCTGT  
ATGTGGCTTAGCTGGGTAGTGTCTTCAACTCCGTGCGGCGCCCCCTTCCCCACCGTGTGTGTGTGTGTGTGT  
TGT  
GTTTTCTGT  
CCTCAGTTGGGCTTGGCCACCTCATAGGATCATCTGCACTTTGCAAAACATAAAGGCCCGCTTTGTAGTTATTTG  
AGCATGT  
CTTGT  
GCTCTGAGTGTGAGGGGCCAGGT  
CAGCATTAGCCGCCAGCCTCCCTCCGCCCCACAGACCCCGCCTGCTGGACCCAGGTGACTTACGCTCCTGGTGG  
GGGCGGGCGGGGAGGGCGGCTTTGCCATCTTGGGGTGGGGGCACTTGCCCTGGGGGCTGGACGTTGGGGGCGGG  
CAGGATTGAGATGGGGCGGGGGTGGGGTCTGGATGGAGGTGGCTGAGCTGGGCGGGGATGGCTCAGGCATGGCT  
GGGATAGATGGGGCTGGGCGGGGCGAGGGGAGGGGCTGGGTGGGACGAGGGGAGGGTTTGGGCGGGGCAAGGCTGGG  
GCTGGGCGGATCTGAGTTGTCCCGAAGGCCCGGAGCTCTGACCTCAGACGCCCTCTTGAACGGCTTTTCCC  
ACTCCTCCCTTTCTAAACGAAGATGCGGCTGGGGGCTTCCCTTCAACGAGGGATCGAGGGCCGCGGGCGAGCA  
CTGAGTCGGATCCCTGGCTCTGGGGCCAGGCCAGGCTTGGCCCGCTGATAGACCTCGAAGATGGCCATCATCTTT  
CTCGTTACCTCAGTCTCTTGGCTCGGGGCCCCAGGGAACGACCTGGTCTCCGGCATCGGATGGGACCGGGGGG  
CGGGGAGGGGTGAATGGGCGAGTGAATTTGAAGAGGGGTGCGGAGGCTGGGCGATGAGGCGGGCTGTCTCACCGC  
TCCCGCAGACAGCGTGGACGATGAATTTGAATTTGTCCACCGTGTGTACCGGCTGAGGGTCTGGAGCAGCTGCAGG  
AGCAAACCAATTACGCGCAAGGAGTTGCAGGTCTGTACCGGGCTTCAAGAACGTGAGTGCNGGGCGAGGCCAA

Fig. 22

ACTCAGCGNGGGTGGGACAGGAGGACCCAANCCGGTCCANATTTTCCCANAAGCATGGCTTNGATGCTTGAGGNG  
CGGGCGGAAGGGAGGCAAGGCCCTGAGACTGAACTTCTAGCTGGAGGTTCTGGGGCGGGGCCAGAACGRAAGTGGCG  
CCTGTAGACTGTCACTTTCTGTTCCATGTTTTTTATTTGTGCACTGGGAAAAGAAGTCTTCCCTCCCATCACATGAGCC  
ACGTGGTGAGTCTCTGGAGGCTTGAAGATTATCCCCCTCCCTGGGAGTCTTGGGCCATGGAGGGTGGGGCGGTGA  
ACGGAAGGGGATTTTGTCTCTGCCCCCAGCCTGGTGCCCTCTCCTTCCAGGAATGTCCAGCGGAATGTCAATGAG  
GAGAACTTCAAGCAGATTACTCCAGTTCTTTCTCAAGGAGGTGAGGGGACAAGGCCCAAGGGGAAGCAGTTGTC  
CTTCTCTAGGCTGAGGGAGGGAGGGATTCTGGAGGAGCTGGGAATGCCAAGGTGATGGGGGGTATGGGGAGCTCCTT  
AGAGGGAGGAAGTCTCTCTGTGTGGAAGCAACTTCTCCACACTCACCTGCAGACTCCAGCACCTATGCCACTT  
TTCTCTCAATGCCCTTTGACACCAACCATGATGGCTCGGTCACTTTTGAAGTGAGCTGGGCGAGGTGGGCCAGGGAA  
GCCTGTTTCTGGAGTTCAGGGCCAGGATCTCCAGGCCAAACCAGAGAAGGAGTTGGGTGAAGAGKACCCGAGGAC  
ACAGCTCCCTNCTGCCTTCTTCCAGGACTTTGTGGCTGGTTTGYCCGTGATTCTTCGGGGAAGTGTAGATGACAGG  
CTTAATTGGGCTTCAACCTGTATGACCTTAACAAGGACGGCTGCATCACCAAGGAGGTGCAGGGCAACTGAAGGGC  
TGGGGGTCTGTGGCGGTGATGGGGGTGGCGTGCAKAGGGTGATGGGAGGGAAATATGACCCACATATGCCACAAGC  
AATGGGATCAAGGGAGGCTGGAGGCTCTGAGGAAGGATCCTCTCTCTTGGCCTAACAGGAAATGCTTGACATCA  
TGAAGTCCATCTATGACATGATGGGCAAGTACACGTACCCTGCACTCCGGGAGGAGGCCCAAGGGAACACGTGGAG  
AGCTTCTTCCAGGTACTTGGGAGTGGGTATGGCTGGAGGGCCCTGGAGTGAAGGGAAGAAGGCCAAGAACCAGCAGG  
GAACTCACCTGACTTCTGTCTGCCCTCTCTTGGCATCCCTCCTGTTCTCCCTGCCGTGACCACCTTCTTGCAAGA  
TGGACAGAAACAAGGATGGTGTGGTGACCATTTAGGAATTCATTGAGTCTTGTCAAAGGTACAGCTCCCTGCCCTC  
TACATTACCCTGACCTGGACTCAGGCCTGATTTAGTAATGCAGGGAAGGCTTCTTTGGGAAGAATACCACCTTCCC  
ACCTCACCCCATATTTCAATCCTATTCCTTTGTGGGAGGCTTACCCCTTCCCTACCTCAGGTCTCTCTGGGCATCT  
CCTTCTCTGTGCTTTTGAATGTCCCGTCTGTGACTCAAGTGTCCCTCTCACTGTCTCTGATAAAGCTCCTTCTCT  
TTCTCTCTCTTCAATCTGCCTCGCTCACATCATGGCCACAGGATGAGAACATCATGAGGTCCATGCAGCTCTTTGAC  
AATGTCATCTAGCCCCCAGGAGAGGGGTGAGTGTTCCTTGGGGGGACCATGCTCTAACCTTAGTCCAGGGGACCT  
CACCTTCTCTTCCCAGGTCTATCCTCATCCTACGCCCTCCCTGGGGCTGGAGGGATCCAAGAGCTTGGGGATTCTAG  
TAGTCCAGATCTCTGGAGCTGAAGGGGCCAGAGTGGGCGAGAGTGCATCTCGGGGGGTGTTCCCAACTCCACCAG  
CTCTCACCCCTTCTCTGCCTGACACCCAGTGTGAGAGTGGCCCTCCTGTAGGAATGAGCGGTTCACCCTCCTA  
CCCCTACTCTAGAAAACACTAGACAGATGTCTCTGCTATGGTGCTTCCCCATCCCTGACCTCATAAACATTTCC  
CCTAAGACTCCCTCTCAGAGAGAATGCTCCATTCTTGGCACTGGCTGGCTTCTCAGACCAGCCATTGAGAGCCCTG  
TGGGAGGGGGACAAGAATGTATAGGGAGAAATCTTGGGCTGAGTCAATGGATAGGTCCTAGRAGGTGGCTGGGGT  
GAGAATAGAAGGGCCTGGACAGATTATGATTGCTCAGGCATACCAGGTATAGCTCCAAGTTCCACAGGTCTGTAC  
CACAGGCCATCAAAATATAAGTTTCCAGGCTTGCAGAAGACCTTGTCTCCTTAGAAATGCCCCAGAAATTTCCAC  
ACCCTCCTCGGTATCCATGGAGAGCCTGGGGCCAGATATCTGGCTCATCTCTGGCATTGCTTCTCTCTCTTCC  
TGATGTGTTGGTGGTGGTGTGGTGGGGGAATGTGGATGGGGGATGCTTGGCTGATGCCGCAAAATTTTCATCC  
CACCTCCTTGCTTATCGTCCCTGTTTTGAGGGCTATGACTTGAGTTTTTGTTCCTCATGTTCTCTATAGACTTGG  
ACCTTCTGAACTTGGGGCTATCACTCCCCACAGTGGATGCCTTAGAAGGGAGAGGGAAGGAGGGAGGCAGGCATA  
GCATCTGAACCCAGTGTGGGGCATTCACTAGAATCTTCAATCAACCTGGGCTCTCCCCACCCACCCAGATAACC  
TCCTCAGTCCCTAGGGTCTCTTCTTGCTTCAATCTACCCAGAGATGCCCCCTTAGCACACCTAGAGGGCAGGG  
ACCATAGGACCCAGGTTCCAACCCATTGTGAGCAGCCAGCCATGCGGCCACCCCTTAGCACACCTGCTCGTCCCA  
TTTAGCTTACCCTCCAGTTGGCCAGAATCTGAGGGGAGAGCCCCAGAGAGCCCCCTTCCCATCAGAAGACTGTT  
GACTGCTTTGCAATTTGGGCTCTTCTATATATTTTGTAAAGTAAGAAATATAACCAGATC:TAATAAAACACAATGGC  
TATGCACAGGCTGCCGTCTCTGCCTTTTGTCCCTCCACCTACAAATACTACACAACCCCTAACGAATGCACCTGCA  
GCCTTTTAGATCCCCAAGAAAGTGGCTTTCTTTTCCATAGTTGGCCATACCTTGGCATGAGACTGAGACACAGGCTC  
TGGAAATGGTTGGAAACCCACCCAACTCAGGCCCCACATGAATCTCCCTCCACACAGCCTGAGAGGAGACAAGGA  
AGGAAGGACAGGACACTGATGTCCCGAAGACTGTGCCAAGCAAGCTGTTTTTAGCTGACATTCTTACAAGTTGAAT  
CACAGATTTCTAATTTACAGACTTTTTAGTTAATCTCAAAGTGCTTTCTTTTGGGGGCTCCTTTAAGTTCYTTCT  
TTTTTTTTTTTTTT

Fig. 22 Continued

>monkey KChIP4 cds = 265

gtcgacccacgcgtccggtgcgctgtggagcgggggggagcccccgcagccaaatgccaggatcagcatgagaggctgg  
acttttagtcaggtctgtcctcacccegggggaccgcggctttgcagggtgcagctgcgaggaactgtcacttttttc  
cccttgcaagtcctttgttccaagcctgacgttgctacgattctgtaattaactccctccactccaaggggtctggaggc  
tgggatgctctgccagctcagaggATGTTGACTCTGGAGTGGGAGTCCGAAGGACTGCAAACAGTGGGTA  
TTGTTGTGAT

TATATGTGCATCTCTGAAGCTGCTTCATTTGCTGGGACTGATTGATTTTTCGGAAGACAGCGT  
GGAAGATGAACGGAGA

TGGCCACTGTCAAGCATCGGCCCTGAGGCCCTTGAGCTTCTGGAAGCCCAGAGCAAATTTACC

AAGAAAAGAGCTTCAGATC

CTTIACAGAGGATTTAAGAACGAATGCCCCAGTGGTGTGTTAATGAAGAAACCTTCAAAGA

GATTTACTCGCAGTTCTT

TCCACAGGGAGACTCTACAACATATGCACATTTTCTGTTCAATGCGTTTGATACGGACCACA

ATGGAGCTGTGAGTTTCG

AGGATTTTCATCAAAGGTCTTTCCATTTTGTCTCCGGGGACAGTACAAGAAAACTCAATTGG

GCATTTAATCTGTATGAT

ATAAATAAAGATGGCTACATCACTAAAGAGGAAATGCTTGATATAATGAAAGCAATATACG

ACATGATGGGTAAATGTAC

ATATCCTGTCTCTCAAAGAAGATGCACCCAGACAACACGTCGAAACATTTTTCAGAAAATGG

ACAAAAATAAAGATGGGG

TTGTTACCATAGATGAGTTTCATTGAAAGCTGCCAAAAAGATGAAAACATAATGCGCTCCATG

CAGCTCTTTGAAAAATGTG

ATTAAacttgtcaactagatcctgaatccaacagacaaatgtgaactattctaccacccttaaagtcggagctaccactt  
ttagcatagattgctcagcttgacactgaagcatattatgcaacaagctttgttttaataataaagcaatccccaaaaga  
tttgagtttctcagttataaatttgcatcctttccataatgccactgagttcatgggatgttctaactcatttcatactc  
tgtgaattattcaaaataaccagatctggcatatagttttattgattccttagccatgggattattgaggctttcacata  
tcagtgatttttaaaataaccagtggttttgcctcattttgtatgtattcagtcctaggattttgaatggtttttctaata  
actgacatctgcatttaatttccagaaattaaattaattttcatgtctgaatgctgtaattccatttatatactttaagt  
aaacaaataagattactacaattaacacatagttccagtttctatggccttcccttcccacttctattataaattaat  
tttatctggtatttttaaacatttaaaaatttatcatcagatatcagcatatgcctaattatgcctaataaacttaata  
agcatttaattttccatcacattatagccaaggcctatatactatatataattttggatttggttaattcttacaggct  
gttttccattgtatcatcaagtggaggtcaagacggcatcaaacaaaacaggatgttacagacatatgcaaagggtc  
aggatatctatcctccagtatatgttaatgcttaataacaagtaatcctaacagcattaaaggccaaatctgtcctctt  
ccctgacttccctacagcatgtttatattacaagccattcagggaacaaagaaaccttgactacccactgtctactagg  
aacaacaaacagcagcaaaattcactttgaaagcaccagtggttccattacattgacaactactaccaagattcagta  
gaaaataagtgtcacaactaatccagattacaatatgatttagtgcataaaaattccaacaattcagattattttt  
aatcatctcagccacaactgtaaagttgccacattactaaagacacacacatcgtccctgtttttagaaaatatcaciaa  
gaccaagaggctacagaaggaggaaatttgcaactgtctttgcaacaataaatacaggtatctattctggtgtagagatag  
gatgttgaaagctgccctgctatcaccagtgtagaaattaagagtagtacaatacatgtacactgaaattttgccatcgcg  
tgtttgtgtaaaactcaatgtgcacattttgtatttcaaaaagaaaaataaaaagcaaaataaaatgttwawaamwwaaa  
aaaaaaaaaaaaa

>monkey KChIP4

MLTLEWESEGLQTVGIVVLIICASLKLHLHLLGLIDFSEDSVEDELEMATVRRRPEALELLEAQSKFT

KKELQILYRGFKNE

CPSGVVNEETFKEIYSQFFPQGDSTTYAHFLFNAFDTDENGAVSFEDFIKGLSILLRGTVQEKLNW

AFNLYDINKDGYIT

KEEMLDIMKAIYDMMGRCTYPVLKEDAPRQHVETFFQKMDKNKDGVVTTIDEFIESCQKDNIM

RSMQLFENVI

Fig. 23

>monkey KChIP4 C terminal splice variant cds = 265-966

```
gtcgacccacgcgtccggtgcgctgtggttgcggtgggggagccccgccagccaaatgccaggatcagcatgagaggctgg
acttttagtccaggtctgtcctcaccgccggggaccgccggctttgcagggtgcagctgcgaggaactgctcacttttttc
cccttgcaagtctttgttccaagcctgacgttgctacgattctgtaattaactccctccactccaaaggggtctggaggc
tgggatgctctgccagctcagaggATGTTGACTCTGGAGTGGGAGTCCGAAGGACTGCAAACAGTGGGTA
TTGTTGTGAT
TATATGTGCATCTCTGAAGCTGCTTCATTTGCTGGGACTGATTGATTTTTCGGAAGACAGCGT
GGAAGATGAACTGGAGA
TGGCCACTGTCAGGCATCGGCCCTGAGGCCCTTGAGCTTCTGGAAGCCCAGAGCAAATTTACC
AAGAAAGAGCTTCAGATC
CTTTACAGAGGATTTAAGAACGAATGCCCCAGTGGTGTGTTAATGAAGAAACCTTCAAAGA
GATTTACTCGCAGTTCTT
TCCACAGGGAGACTCTACAACATATGCACATTTTCTGTTCAATGCGTTTGATACGGACCACA
ATGGAGCTGTGAGTTTCG
AGGATTTTCATCAAAGGTCTTTCATTTTGTCCGGGGGACAGTACAAGAAAACTCAATTGG
GCATTTAATCTGTATGAT
ATAAATAAGATGGCTACATCACTAAAGAGGAAATGCTTGATATAATGAAAGCAATATACG
ACATGATGGGTAAATGTAC
ATATCTGTCTCTCAAAGAAGATGCACCCAGACAACACGTCGAAACATTTTTTCAGGCTGTTT
TCCATTGTATCATCAAGT
GGAAGTTCAAGACGGCATCAAAACAAAACAAGGATGTTTACAGACATATGCAAAGGGTCAGG
ATATCTATCCTCCAGTATA
TGTTAAtgcttaataacaagtaatcctaacagcattaaaggccaaatctgtcctctttccctgacttccttacagcatg
tttatattacaagccattcagggacaaagaaaccttgactaccccactgtctactaggaacaaacaaacagaaagcaaaa
ttcactttgaaagcaccagtggttccattacattgacaactactaccaagattcagtagaaaaataagtgtcaacaacta
atccagattacaatatgatttagtgcataaaaattccaacaattcagattattttaatcatctcagccacaactgta
aagttgccacattactaaagacacacacatcgccctgtttgtagaaatatcacaagaccaagaggctacagaaggag
gaaatttgcaactgtccttgcaacaataaatcaggtatctattctggtgtagagataggatgttgaaagctgccctgcta
tcaccagtgtagaaattaagagtagtacaatacatgtacactgaaatttgccatcgcggtgttggtgtaaactcaatgtgc
acattttgtattttcaaaaagaaaaataaaaagcaaaataaaaatgttwawaamwwwwaaaaaaaaaaaaaaaaaaaaa
```

>monkey KChIP4 C terminal splice variant

```
MLTLEWESEGLQTVGIVVIICASLKLHLGLIDFSEDSVEDELEMATVRRRPEALELLEAQSKFT
KKELQILYRGFKNE
CPSGVVNEETFFKEIYSQFFPQGDSTTYAHFLNFDTDENGAVSFEDFIKGLSILLRGTVQEKLNW
AFNLYDINKDGYIT
KEEMLDIMKAIYDMMGKCTYPVLKEDAPRQHVETFFQAVFHCIIKWKFKTASNKTRMFTDICK
GSGYLSSSIC
```

Fig. 24



```

KCHIP1_1v -----MGAAMGTF-----SSLOTKQ-----RKP-----
KCHIP2_9q1 MRGQGRKESLSRDLDCSYDQETGHPGPTKKALKORFLKLLPCCGPQALPSVSETLAA
KCHIP3_p19 --MQPAKEVTKAS---DCSITGDLGH---TPLSKKEGLKWQRPRLSRQALMRCCLVKWI
KCHIP4_352 ---MLTGEWESEGLQTVGIVVILECAS---LKLHLHLLGGEDFSE-----
KCHIP4_231 ---MLTGEWESEGLQTVGIVVILECAS---LKLHLHLLGGEDFSE-----
hsncspara ----HEVTSISAQLEEASTGGFLYAQN-SIKRSIKERIMKLLPCS-----

KCHIP1_1v -----SKDKIEDELEMTMVCHRPEGLEQLEAQTNETKRELOMIVRGEKNECPES
KCHIP2_9q1 PASLRPHRPRLLDPSVDEFEFESTVCHRPEGLEQLEQEQKRETRKELOMIVRGEKNECPES
KCHIP3_p19 LSSTAPQ-----GSDSSSESTIEESTVRHOPEGLEDQLEAQTNETKRELOMIVRGEKNECPES
KCHIP4_352 -----DSVEDELEEMATVRHRPEAGELLEAOSKEETKRELOMIVRGEKNECPES
KCHIP4_231 -----DSVEDELEEMATVRHRPEAGELLEAOSKEETKRELOMIVRGEKNECPES
hsncspara -AAKTSSP---AIQNSVDEDELEEMATVRHRPEAGELLEAOSKEETKRELOMIVRGEKNECPES

KCHIP1_1v FVANDPTTKELLSQEPPEGDSSTTAHLEENARDTDHNGAVSPEDETKGLSILIRCTWQEK
KCHIP2_9q1 FVANDPTTKELLSQEPPEGDSSTTAHLEENARDTDHNGAVSPEDETKGLSILIRCTWQEK
KCHIP3_p19 FVANDPTTKELLSQEPPEGDSSTTAHLEENARDTDHNGAVSPEDETKGLSILIRCTWQEK
KCHIP4_352 FVANDPTTKELLSQEPPEGDSSTTAHLEENARDTDHNGAVSPEDETKGLSILIRCTWQEK
KCHIP4_231 FVANDPTTKELLSQEPPEGDSSTTAHLEENARDTDHNGAVSPEDETKGLSILIRCTWQEK
hsncspara FVANDPTTKELLSQEPPEGDSSTTAHLEENARDTDHNGAVSPEDETKGLSILIRCTWQEK

KCHIP1_1v ERITTEFTNLSSTANKSGALENKTEEMMDLWKALEDMMKKYVLPALKEETPRORHVDVEKQKME
KCHIP2_9q1 ERITTEFTNLSSTANKSGALENKTEEMMDLWKALEDMMKKYVLPALKEETPRORHVDVEKQKME
KCHIP3_p19 ERITTEFTNLSSTANKSGALENKTEEMMDLWKALEDMMKKYVLPALKEETPRORHVDVEKQKME
KCHIP4_352 ERITTEFTNLSSTANKSGALENKTEEMMDLWKALEDMMKKYVLPALKEETPRORHVDVEKQKME
KCHIP4_231 ERITTEFTNLSSTANKSGALENKTEEMMDLWKALEDMMKKYVLPALKEETPRORHVDVEKQKME
hsncspara ERITTEFTNLSSTANKSGALENKTEEMMDLWKALEDMMKKYVLPALKEETPRORHVDVEKQKME

KCHIP1_1v ---KNKRGVLDLHLEFESCKDENMRSLOKQNM
KCHIP2_9q1 ---KNKRGVLDLHLEFESCKDENMRSLOKQNM
KCHIP3_p19 ---KNKRGVLDLHLEFESCKDENMRSLOKQNM
KCHIP4_352 ---KNKRGVLDLHLEFESCKDENMRSLOKQNM
KCHIP4_231 IKWKKKTAANKTRMTDICKGSGYSSSIC-----
hsncspara -----

```

Fig. 25

## Rat 33b07 protein

MNGVEGNNELPLANTSTLSALVPEDLDLQDQPLSEETDVTREMEAAAGEAGAEGGASPDSEHCDPQLCLRVAENGCAAAAG  
EGLEDGLSSSKCGDAPLASVAANDSNKNGCQLAGPLSPAKPKTLEASGAVGLGSQMMPGPKTKVMTTKGAISATTGKEG  
EAGAAMQEKKGQKEKKAAGGGKDETRPRAPKINNCMSLEAIDQELSNVNAQADRAFLQLERKFGMRRLHMQRFSFII  
QNIPGFVWTAFRNHPQLSPMISGQDEDMRYMINLEVEELKHPRAGCKFKFIFQSNPYFRNEGLVKEYERRSSGRVVSLS  
TPIRWHRGQEPQAHHRNREGNTIPSFNWFSDHSLLEFDRIAELIKGELWSNPLQYYLMGDGPRRGVRVPPRPVESP  
SFRFQSG.

## Rat 33b07 DNA (coding: 85-1308)

GGTGGAGCTAAGCACTCACTGCGGTGCTGCCCTGCGTCTGCAGAGAAACAAGGAAAGCTTCTCTGCAGGGCTGTCAGCTGC  
CAAAATGAACGGCGTGAAGGGAACAACGAGCTCCCTCTCGCTAACACCTCGACCTCCGCCCTTGTCGCGGAAGATCTGG  
ATCTGAAGCAAGACCAGCCGCTCAGCGAGGAACTGACACGGTGCAGGAGATGGAGGCTGCAGGTGAGGCCGTGCGGAG  
GGAGGCGCTCCCCGATTCGGAGCACTGCGACCCCCAGCTCTGCCTCCGAGTGGCTGAGAATGGCTGTGCTGCCGAGC  
GGGAGAGGGGCTGGAGGATGGTCTGTCTTCATCAAAAGTGTGGGGACGCACCTTGGCGTCTGTGGCAGCCAACGACAGCA  
ATAAAAATGGCTGTGAGCTTGCAGGGCGCTCAGCCCTGCTAAGCCAAAACTCTGGAAGCCAGTGGTGCAGTGGGCTG  
GGGTGCGAGATGATGCCAGGCGCPAAGAAGACCAAGGTAATGACTACCAAGGGCGCCATCTCTGCGACTACAGGCAAGA  
AGGAGAAGCAGGGGCGCAATGCAGGAAAAGAGGGGGTGCAGAAAAGAAAAAAGGCAGCTGGAGGAGGAAAGACGAGA  
CTCGTCTTAGAGCCCCCTAAGATCAATAACTGCATGGACTCCCTGGAAGCCATCGATCAAGAGCTGTCAAATGTAAATGCG  
CAAGCTGACAGGGCCTTCCTCCAGCTGGAACGCAAAATTTGGGCGGATGAGAAGGCTCCACATGCAGCGCCGAAGTTTCAT  
CATCCAAAACATCCCAGGTTTCTGGGTCAAGCGTTTCGGAACACCCGCAACTGTCAACGATGATCAGTGGCCAAGATG  
AAGACATGATGAGGTACATGATCAATTTAGAGGTGGAGGAGCTTAAGCACCACAGAGCAGGGTGCAAATTTAAGTTCAATC  
TTCCAAAGCAACCCCTACTTCCGAAATGAGGGGCTGGTCAAAGAGTACGAGCGCAGATCCTCAGGTGAGTGGTGTGCGT  
CTCTACGCCAATCCGCTGGCACCAGGGGTCAAGAACCCAGGCCCATATCCACAGGAATAGAGAGGGGAACACGATTCCCA  
GTTTCTTCAATTTGGTTCTCAGACCACAGCCTCCTAGAATTCGACAGAATAGCTGAAATTTATCAAAGGGGAGCTTTGGTCC  
AATCCCCCTACATACTACCTGATGGGCGATGGGCCACGCAGAGGAGTTCGAGTCCACCAAGGCAGCCAGTGGAGAGTCC  
CAGGTCTTTCAGGTTCCAGTCTGGCTAAGCTCTGCCCTCGTGAGAAGCTCTTACAGAAGAGTCTTACCACCTTCTCAGC  
TTGGCTAGCAGCATGCAGCCTTCTGTCTGCTTTCTCTTCTTGGATTGTGTCCTTTGGTTCTTCTAAGTCTCCGGTAGTT  
TCAAGGTTGTGGCTTCCAAGTCTTTGCTCTTCTTCTTCTTGGCCATCACGATGTCTGCATAGTGTAAATGGTGTTCAA  
GTGCTGATGCTTCTAGGTTTTTTGTTTTCTTTTTTAAAAGTGGTTCTCTATCAAAGAAAGCTTGACATATCCTTACCAA  
GAACTAGCCAGATTTCTACTGTGTTCCCGATATCTATGTACTGTGAAGAACTGTGAGTTTCCGCACTGCAAGATGGGAC  
TGATATCCCAATCCAGCCATCAGCCCAACAGGACATTCCAAGCTGTACCAACTGATCCTAGCTGTCTTCTGGGCTTTG  
CCATTTACCCTGCTTTTTATCTATAGAAAGAGCAGGTGGCTGGTAGGTGACTACTAGGTAAGAGTGAAGTATTAGGTGAG  
GAGTGTCTTCTGTACCAACATTGTTCTTGTACCAATGCATCATGATCAGCTTGGATCAGCTACTGACTGTCTGATATTTT  
TAACCCCCAACACAAAAA

Fig. 26

Human 33b7 (106d5) DNA (coding: 88-1332)

GGGGTGGTGCCTAGACGTTTCGGGCGAGAGCTCGGCCCTGCGGAGGACAAGGAAGTCTCCCTCTCCCACTAGTCTGACTTC  
 TTCCAAATGAGCGGCTGGATGGGGGCAACAAGCTCCCTCTCGCCCAAAACGGCGGCTGGCTGCTCCCGACCATGCCCT  
 CAGGAGATCCGGACCTAGACAGTGCCTAAGGGCTCCGTGAAGAAACCGAGGCGACACAGGTGATGGCGAACACAGGTGGG  
 GGCAGCCTGGAGACCGTTGCGGAGGGGGGTGCATCCAGGATCCTGTGACTGTGGCCCCGCGCTCCGCGTCCCAGTTGC  
 CGGGAGTTCGGGGCGGTGCAGCGACCAAGCCGGGCAAGGAGGATGCTCCACCTTCTACGAAAGGTCTGGAAGCAGCCTCTG  
 CCGCCGAGGCTGCTGACAGCAGCCAGAAAAATGGCTGTCAGCTTGGAGAGCCCCGTGGCCCTGCTGGGCAGAAAGGCTCTA  
 GAAGCCTGTGGCGCAGGGGGCTTGGGGTCTCAGATGATACCGGGGAAGAAAGGCCAAGGAAGTGACGACTAAAAAACGCCG  
 CATCTCGGCGCAGTGGAAAAGGAGGAGAAGCAGGGGCGCGATGGAGGAAAAAGAGGTAGTGACAGGAAAAAAAGG  
 TGGCAGGAGGGGTGAAAGAGGAGACCGGCCAGGGCCCCGAAGATCAATAAAGTGCATGGACTCAGTGGAGGCCATCGAT  
 CAAGAGTTGTCAAACGTAAATGCCAGGCTGACAGGGCCTTCTTCAGCTTGAGCGCAAGTTTGGCCGATGCGAAGGCT  
 CCACATGCAGCGCAGAAAGTTTCATTATCCAGAATATCCAGGTTTCTGGGTACTGCTTTCGAAACACCCCCAGCTGT  
 CACCTATGATCAGTGGCCAAAGATGAAGACATGCTGAGGTACATGATCAATTTGGAGGTGGAGGAGCTTAAACACCCCA  
 GCAGGCTGCAATTCAGTTTATCTTTCAGGGCAACCCCTACTTCCGAAATGAGGGGCTTGTCAAGGAATATGAACGCAG  
 ATCCTCTGGCCGGTGGTGTCTTTCCTCACTCCATCCGCTGGCAGGAGGCCAAGACCCCAAGGCTCATATCCACAGAA  
 ACCGGGAAGGAAACACTACTATCCCTAGTTTCTTCACTGGTTTTCACAAATAACTGATGGAGCACAGCCTTCTAGAATTG  
 CAGACGAG  
 ATTATCAAAGGAGAACTGTGGCCCAATCCCTACTACTACCTGATGGGTGAAGGGCCCCGTAGAGGAATTCGAGGGCCC  
 ACCAAGGCAGCCAGTGGAGAGCGCCAGATCCTTCAGGTTCCAGTCTGGCTAATCTCTGTCTGTGAGAAGCTTCTGCACA  
 AGTTTCCTTACCACCTCCTCTTGGACCTATGCTTGGCCAACAGCATGCAGTCTTCCATCTGCTTTCTCTTCATACTGTGG  
 ATTATCTTTTCTTTGGTTCTAAATCTTCAGTAATCGGTTGCAAGATTGTGGCTTACCTGCTGTGCCATTCTTCTCTCT  
 GGGCCTTCATGCTTTTCTGCATTGTGTAAATGTTTCAAGTGCATGGCCTTCTACGGCTTCTATGCCAAGCGTATGATA  
 CTATAGATATAGTGTACCATCTGCTTTCTTTCATGGCTTGGACCTATCTGTGACCATGCTCTTCTCCCAATTTAAG  
 TGGTTCTGTACCAAAAGAACTCTGTATACATTTTACAAATAACTGATGGCTTCTATGCTGGCTTCTATGCTGGCTGTGCTC  
 ATACCCATGTACTTATGGTAAGCTATTTGGGTATTACCACTGCAAGACAAAAGTATATCTTAAACCCGGCCATCAACCCA  
 AATTGGACATTCCAGACTACCACCACTGGATCCAGCTGCCTTCTGGGCTTGTGCCATCCACCTACTGGTTATCTGA  
 TAGAACAAGCTGGTGGCTGATGGGTGACTGCTAGGCGTACTGAGGTAATAGATGAAAAGTGTCTATGTTATCACATTG  
 GTTTTCTGTACCTTTGGTTACTCTACGTACATGACAGCTGCTGGTGAGTATGAAGCCTGTGCTATAGCCCAACCCCTACT  
 CACTCTCACTTCTGGTTGAACCTTGGCTTAGGCCACCATTTGTCTGCCTCATCAGGAATATCTGTAGACGTAGCTCCAG  
 GGAGCTCACAGCAACACCCCTTACCACCAAGATGGGCAGTAATATGTGACAGAGCCCAAGCAAGGCTGGAACGCAAGTCC  
 CTTCAGCTTATGCTTTCTGACTCTTACCAACCAAGCAAGCTTAAATGTGAGCAACTTCTTTAGGCATTTCTCTTTTCC  
 CCGCTGCACCCACTCTGTAAGTATGCAAAAAGTTGCCAGAGTTGGGGCATTGAGGAAGAGATATTTCTGGAATGTGAGACT  
 TGTATGCTCTGTCTCTTTCTCTCCCTCCCTCTCCCTCTCCCTCTCCCTCTCCCTCTCCCTCTCCCTCTCCCTCTCCCTCT  
 CTCTGAAGCAGTTTCTAGCTTATTAACAGAAAACAAAAGTGGCAAGCAGGCTTTTGTGTTAATTTGCTCTTTCCCTGATT  
 GTGTTGAGAGAGAAAGTTATGATTAAATGGGCTCCAGATCTCTTATTGCCCTTATTTCTCCACCCCACTTCTTTAGCA  
 AGGTCTGAAAGTTTCAAAGGGAGACCTATAGGTTAATGTTTATGTTATAGGCAGTGTAAATTAGGCAGATTGTGACATA  
 TTTATCTTTTATACCCATCCATTCTACCAAAACCTGTGTTATTTCTTGTAGTTTGTAGTTTGTAGTTTGTAGTTTGTAGTT  
 AGGCTCTTCAAGTGTAGTTTCAAGGACGGGTCAAAGCCAAAGGCTTGTGATGTGAGCAAGGCAACCAAAAGTGTAGCC  
 TCAGTCCACTTTTCTAAAGATGGAATCTTTTGGGGCTTGGACTGCTTCTAGGGTAGCATTTGTAGGTCACTCTTC  
 TCCTTGTACTATTTTGTGCT  
 AGCTTCTGCAATTTCTTCCCTGCTCCTAACAAATGAAGAAGCAGGCTGCAGCCTGCATTGTGGAAGATCTCCAGCCTCCT  
 TGTAGGGGATAAGGGGATGTGTAGCATCTGTGTGGATTTCACGGAACAAGTTCAGTAGGTGGGACAGTGATCCGTCAA  
 GGCTTAGTTATGATCATGTGTGGTGTAAAGACCATCCACCATCAGCCTTTTCCCTTTGTTTGAAGGCGCTGCTGCTGCT  
 AGCTACCTGAGGGTTTAGGAGGTCTGAACACACAGTGGAGAGGTTAATCTAGGTGGGAACTGAGTAAAGTCCAGA  
 GCAGGAATGAGCCTGCTGTGGCGTGGGTTTGGAAAGGCTCACAGGAAGAAGCTGCAGGATCAGGGGTGGGAGGGGAGGC  
 CCCTGAGGTGCTCTCCAGGAAGAGGGGCTGGGGTTAAATAGCATGCTTGGAGGAAGATTTTCTTCAATTTTCTTAA  
 GTCCTTGAATTCACCACTAGATTTTTGTAAACAAAATGTAAGTCGATGTTTTCTCTCAATTATCCTAGGAGTGACCTTTA  
 TATGTGTGGAAGATTAAATGTTATATGCTCCTTATGTCACTGTTTTTGTAGTAAATCCATTTCTTCTCTGTTTACGCTT  
 ATGACAAAATGATGTTTACAGGCCTGCTTTTGTCTTATAATGACAACATGTGCAAAAATACCAAAATTTGTGCTCCTGTG  
 CAGTATGAAGAATTCAGTGAATATTATTAATGTTATGCTGTTTTGCTCTCTGTTTATATATGCTCTATTCTTAGAA  
 ATATAATTTGAATGTGATCTTTCAATAGTCTGAATATTTTACAAATATATAGCTATGCTTGTGAAAATAACCTCAAAAAG  
 AAAAATACGACTCTGTTGCTTACTTGATATTTCTTGGCCTAGTAATGTACTTGACATTATGTTTCTTAAGCAGTGTAAAG  
 TACCAGTAGAATTTCTCTGTCAAACCTCAATGATCATTTAGTACTTTTGTCTTCTCCATGTGCTTGAAGGAAAAATAAG  
 TGTCACTACCGTATTTCTGTTTTTCATCAAAAATAAAAATAATTTAAAAAACAAAAA

Human 33b7 (106d5) protein

MSGLDGNGKLP LAQTGGLAEPDHDGDPDLQDQGLRETEATQVMANTGGGSLETVAEGGASQDPVDCGPALRVFVAGS  
 RGGAATKAGQEDAPPSTGLLEAASAAEAADSSQKNCQQLGEPRGPAGQKALEACGAGGLGQSMIPGKKAKEVTTKKRAIS  
 AAVEKEGEAGAAEKKVQKEKKVAGGVKEETRPAPKINNCDMSLEAIDQELSNVNAQADRAFLQLERKFRMRRLHM  
 QRRSFIIQNIPIGFVWTAFRNHPQLSPMISGQDEDMRLRYMINLEVEELKHPRAGCKFKFIFQGNPYFRNEGLVKEYERRSS  
 GRVVSLSSTPIRWHRGQDPQAHIRNREGNTIPSFNWFSDHSLLEFDRIAEIKGELWPNPLQYYLMGEGPRRGIRGPPR  
 QPVESARSFRFQSG

Fig. 27

## Rat 1p protein (partial)

LKGARPRVNSTCSDFNHGSALHIAASNLCGLAAKCLLEHGANPALNRKQVPAEVVDPMDMSLDKAEALVAKELRT  
LLEEAVPLSCTLPKVTLPNYDNVPGNMLLSALGLRLGDRVLLDGQKTGTLRFCTTEFASGQWVGVELDEPEGKNDGSVG  
GVRYFICPPKQGLFASVSKVSKAVDAPSSVTSTPRTPRMDFSRVTGFRREHKGKKKSPSSPSLGSLLQQRZGAKAEVGD  
QVLVAGQNRDCAFLWEDRLCSRLLVWH

## Rat 1p DNA (partial, coding:1-804)

CTGAAAGGGGCGAGGCCAGGGTGGTGAACCTCCACCTGCAGTGACTTCAACCATGGCTCAGCTCTGCACATCGCTGCCTC  
GAATCTGTGCTGGGCGCCGCAAAATGTTTACTGGAGCATGGTGCCAACCCAGCGCTGAGGAATCGAAAAGGACAGGTAC  
CAGCGGAAGTGGTCCCAGACCCCATGGACATGTCCCTTGACAAGGCAGAGGCAGCCCTGGTGGCCAAGGAATTCGGGACG  
CTGCTAGAAGAGGCTGTGCCACTGTCTGCACCTTCTTAAAGTCACACTACCCAACTATGACAACGTCCCAGGCAATCT  
CATGCTCAGCGCGCTGGGCTGCGTCTAGGAGACCGAGTGCTCCTCGATGGCCAGAAGACGGGCACGCTGAGGTTCTGCG  
GGACCACCGAGTTCGCCAGTGGCCAGTGGGTGGGCGTGGAGCTAGATGAACCGGAAGGCAAGAACGACGGCAGCGTTGGG  
GGTGTCCGGTACTTTCATCTGCCCTCCCAAGCAGGGTCTCTTTGCATCTGTGTCCAAGGTCTCCAAGGCAGTGGATGCACC  
CCCCCTCATCTGTTACCTCCACGCCCCGCACTCCCCGGATGGACTTCTCCCGTGTAAACGGGCAAGGCCGGAGGGAACACA  
AAGGGAAGAAGAAGTCCCCATCTTCCCCATCTCTGGGCAGCCTGCAGCAGCGTGAAGGGGCCAAAGCTGAAGTTGGAGAC  
CAAGTCTTGTGGCAGGCCAGAACAGGGATTGTGCGTTTCTATGGGAAGACAGACTTTGCTCCAGGTTACTGGTATGGCA  
TTGAAGTGGACAGCCACGGGCAAGCATGACGGCTCTGTGTTCCGGTGTCCGGTACTTTACCTGTGCCCCGAGGCACGGG  
GTCTTTGCACCAAGCATCTCGTATCCAGAGGATTGGTGGATCCACTGATCCCCCTGGAGACAGTGTGGAGCAAAAAAGT  
GCATCAAGTGACAATGACACAGCCCAACGCACCTTCACAACAGTCCGGACCCCAAGGACATTGCATCAGAGAATCTTA  
TCTCCAGGTTACTCTTCTGCTGCTGGTTTCTTGGATGCTGAGGGCGGAGATGCAGTCTTAGAGACCTGGATACCTGACA  
CAGAGACAGAGTCCCCCTTAGCATCTCTGACACAAGGAGACCCCACTCACCTTAAGATAGAGATTCCCAGTGACACCTC  
CAGAATAGAAAACCCCGTTAGCCAGCCCTCGATTACTGAGGTCCCATTTATTAACAGATCTCCCATGACGACTCCCCCAAAT  
ACAGACCTCATGTTACCCCAAAAAGAGATTCCCTGAGTAGCACCTTCAGGCTAGTCCCTGTCCCTTACCCCTCAGAGCAGA  
TTTCCCCCAATAAACATTTTCCACATCACCCAAGGGATGCTGACCTCTCCACGACAGGACGTTCTTGAGTTACAGTGG  
ATTAGAGTCCCATGAATGAAGACCCCCCCCCACCCCGTTCTCTTAAAGCATAGGTCATACCTCCAGAATAGCCAGCCACA  
TCACTATCCCATGTAACATCAGTCTCTCAAATGGCGTGAGGTCACTAGAAAAGACCTTATACTCTCTCTCTCTCTCA  
GAGATGCCCTCCATTCACTTAAGTCCCTGTTCTCACCCCTGAACAAGACACCTAATTAACCGGCCCACTCACCTCAATTA  
CAAACACCAAAATCGTCTGGAAGCATGAATTACAGGACAGCAAGTCTTCTGCCCCTCTGCACCCCTTGAGAAAACCCCAAG  
TGCCTTGTATGAAGCCCAACCCACATGGCCACAGTCCCTGTGCTGGCCAAGGCTCCAGAAAATTTCTCTATTTTTTAAA  
GTAATAACTTCCCCCCTTTGGGGGGATCCCCAAATTTGGAGACCCCAATTCTAGAACACTGGGGAGTTCAAATTTCCAGAG  
AGAATATATATATATATATAATCCCCAATTTCCCATGCTTCCAAGCCCTACAATCTCTAGAAGACCCCAATTTCTAATTC  
CCAGGACTTCCCCTACCCAAAGTCACAGAATCTTCAAATCCCCAGGGGAATCCCAAACCTTAAGATACCAATCCCAAACCTC  
AGGAAATCCCCCAACACAAGGTCTTAGGACCGGGAGGAAGGAACCTGTTGCCAGGAGAACATCCAGGCTCTCAGGGCA  
TCTCAAACCTGACTCCAGGCACACAGGAGACCCCAACAGAAAGTCCCATCTTTGGAACAAGGATAGGACTCTAATACCC  
TTAGTCCATGGATCTTTAATTTCCCAACCTCCAAACTCCATGGGCCCCACCTCAAGGGAACCCCAAGATCCAAATCTC  
TGATAACTAATATGTGCAGGGCCCCAGGGCTCTAACAGGACCCCAATCATGGAGTCCCTACTTCAATCTACCTTCTGGT  
CACAGGTCCAAGACACTAATCTGAGTCATTGGCCCCAAAGGACTTCACAGCACCTGGGCCAGACTAACAGCTGAGGGA  
GAACCTGAGGGCCCCGTGGGTCCAGAGCAGACCTGGGGCCCTGACCACCAAGGACAGCTCACGACTGCCCTTCACTGCA  
TGTCCCTAAACTCAGCATGACTCTGTCTCTTCAATAAAGACGTTTCTATGGCAAAAAAAAAAAAAAAAAAAAAAAAAA  
AAA

Fig. 28

## Rat 7s Protein (partial)

ADSTSRWAEALREISGRLEMPADSGYPAYLGARLASFYERAGRVKCLGNPEREGSVSIVGAVSPPGGDFSDPVTSATLG  
IVQVFWGLDKKLAQRKHFPVSNWLISYSKYMRLDEYDKHFTEFVPLRTRAKEILQEEEDLAEIVQLVGKASLAETDKI  
TLEVAKLIKDDFLQONGYTPYDRFCPFYKTVGMLSNMISFYDMARRAVETTAQSDNKITWSIIREHMGEILYKLSSMKFK  
DPVKDGEAKIKADYAQLLEDQMNAFRSLED

## Rat 7s DNA (partial, coding: 1-813)

GCTGACTCTACCTCTAGATGGGCTGAGGCCCTCAGAGAAATCTCTGGTCGCTTAGCTGAAATGCCTGCAGATAGTGGATA  
CCCTGCATACCTTGGTGCCCGACTGGCTTCTTTCTATGAGCGAGCAGGCAGAGTGAAATGTCTTGGAAACCTTGAGAGAG  
AAGGGAGTGTCTAGCATTGTAGGAGCAGTTTCTCCACCTGGTGGTGATTTTCTGATCCAGTCACATCTGCTACTCTGGGT  
ATTGTTTCAGGTGTTCTGGGGCTTGGATAAGAAGCTAGCTCAGCGCAAGCACTTCCCGTCCGTCAACTGGCTCATTAGCTA  
CAGCAAGTACATGCGCGCCCTGGACGAGTACTATGACAAACACTTCACAGAGTTCGTGCCCTCTGGAGACCAAAGCTAAGG  
AGATTCTGCAGGAAGAGGAGGATCTGGCGGAAATCGTGAGCTCGTGGGAAAGGCGTCTTTAGCAGAGACAGATAAAATC  
ACCCTGGAGGTAGCAAACTTATCAAAGATGACTTCCACAAACAAATGGGTACACTCCTTATGACAGGTTCTGTCCATT  
CTATAAGACCGTGGGGATGCTGTCCAACATGATTTTCATTCTATGATATGGCCCGCCGGGCTGTGGAGACCACCGCCAGA  
GTGACAATAAGATCACATGGTCCATTATCCGTGAGCACATGGGGGAGATTCTCTATAAACTTTCTCCATGAAATTCAG  
GATCCAGTGAAGGATGGCGAGGCAAGATCAAGGCCGACTACGCACAGCTTCTTGAAGATATGCAGAACGCATTCCGTAG  
CCTGGAAGATTAGAACTGTGACTTCTCTCTCTCTCTCCGAGCTCATATGTGTATATTTTCTGAATTTCTCATCTCCA  
ACCCTTTGCTTCCATATTGTGTCAGCTTTGAGACTAGTGCTCGTGCCTTCGTTTCATTTTGTCTGTTCTTTGGTAGGTC  
TTATAAAACACACATTCCCTGTGCTCCGCTGTCTGAAGGAGCTCCTGACCTTTGTCTGAAGTGGTGAATGTAGTGCATATG  
ATACACAGTGTAAACATACACATTGTAACATATACGTTCTGTAAACTTGTATGTAAGGTGACTACCCCTTCCCTCTCTCC  
AGTAAACTGTAAACAGGACTACTGCATGTGCTCTATTGGGGATGGAAGGCCAGATCTCCATACCGTGGACAGGTACATAA  
GGAAACTAGACCACTTGCAACTTAGTGTTTGTGAGTAAACATTTTGCAGGAAAGTATTTCCATTTAAAAACAAAAGATT  
AATGTTCCAATTTATTTGTAGCTTCCCCAGTATCAATCAGGACTGTTTGTGGCGCACTTGGGAACTATTTTGTMTTCTTAA  
CAGACGTTTGAAGGCTGAACGTAATAGATAAATCAGTTCCCTCTGAAAGTGTGAAAGTAAAAAGAGAGCTAGGTGGTCA  
GACTTAAATTGACATCGTCTTGTTTAAGCATATTTTATTTCACTGAGAGATTTAATATCAAGGACTTTTATATACTCAAT  
TACTAGGAAATCTTTTTTTAAGTACAATTTAAAAATCATTGAAATGTGATCCACATCATAGCCATTTTCTTATATTTA  
GTCAGATGAGCTCAGAGTGGGGAGGGTGTGGGTAGAAATACCAAGGACACGCAGCAGTGCTGCAGGCAGTGTGGCCG  
GGGGCCAGAGCGGCATTGTTTTACAGAGGTACGTGTGTGGCGTGTGTGTTTCTTGTGACACTCTGAAAACAGCAAGCT  
TACCAGTTCACAGGAAATATTTTGTCTTTTCACTGGCTCAGAAAGCTCCTCAAAGTACCTGGTCCCTGAAGCTTCCAT  
CTGTTAATAGAGACGAGAGAGGTTCTTAAATTTAACTGGTGACAAACAAAAAGAAAAAAGATCGATTTTGTCTTGC  
TGTTTTGGTGTGTTTAAATAATAATTCCATATTTGCATAACGAGGCTCGCTTCTGAGAGCTTGGAGATCGTGCTCCCTCT  
TCACTCTCCGGGTGATAATGCTGGCGCCATGCTACCTTTCAGGAGGGGAAGGGGATTGAACATGGCTAACACTCTCAA  
GTACACAAGCGTAAACGACAAAGTATTTATTTAAGCCTTGGTATGTTGTTTAAATTATTAGGTGGTGCATTTCTTATGGT  
CTTTTGGGTAGACATAGTATACACTTCAGATGTAATGTGTAAATCCTTGCTAGTGATGTCTACACGATAGACTGCTATT  
CAAGAAGGATATTCTTCCACATAACAATTTAAAACTATTAAATCAGATATGGATTATGCAATGACTTGTGAGAGGTGG  
ATTAACGGTGTCTTAATCAGTTTGCTTCCAATATGGCTTCGTATCCAGAAGCCCTGACTAGTGGAGATGAGAAAGATT  
TCAAAACCTGTCTGCCTACACCTACCAGCAACCTAGGCTTGTGATCAGAATGAATGATCCCAAGAACTACTTGACCAAG  
TGTGTTTTGTTGCTTGGATTTGAGATGTGCGTTCTTCTCCCTCTGAGACTGTTGATGTATGAGTGTGAAGAGTTACA  
GAAACAACGCTCAGATTTTCACGGTAACTTTCCCTCTGCCCACACTGTAGAGTTTCAGATTGTTCACTGATAGTGCTTCT  
TTCGTAAGGATGTGTTAAATATAGCAGTCTTTTTTAAAGATTATGCAGTTCTCTATTTATTGTGCTGTGCCCTGGTCTTA  
AGTGCAGCCGGTTAAACAAGTTTCATATGTATTTTCCAGTGTAAATCTCATACCTATGCCCTTTGGAAAGCTCCATCC  
TGAACAATGAATAGAAAGAGGCTATATAAATTGCCTCCTTATCTTAAAGATTTCATATCTTTATGTTAAGAGTAATGTAT  
AATTATTAAAACTTATGAAAAATAAAAAGTGGATTAAATTAAGAGATC

Fig. 29

## Rat 29x protein

ARLPAPAHARQQPLLSGPEPGSSARVPVPGVASRRQPRGGKPPSGDGLSEGPSRPLLHARGEAGLHRQSGRVPHTGTAY  
FADEPTEAQAPGGFCVSPSLLGVRWPACATRTPGSLPLSPPSAQPRTLWPTPPAGPSSRMVARNQVAADNAISPASEPRR  
RPEPSSSSSSSSPAAPARPRPCPVVPAPAPGDTHFRTRSHSDYRRITRTSALLDACGFYWGPLSVHGAHERLRAEPVGT  
FLVRDSRQRNCFALSVKMASGPTSIRVHFQAGRFHLDGSRETDFCLFELLEHYVAAPRRMLGAPLRQRRVRPLQELCRQ  
RIVAANGRENLARIPLNPVLRDYLSSFPFQI

## Rat 29x DNA (coding: 433-1071)

GCACGGCTCCCGGCCCGGAGCATGCGCGACAGCAGCCCCCTCCTCtCCGGCCCTGAGCCCCGGATCGTCCGCCCGGGTTCC  
AGTTCCCGGCGTGGCCAGTAGGCGGCAGCCGCGAGGCGGCAAGCCACCCAGCGGGGACGGCCTGGAGTCGGGCCCTCTC  
CACGCCCCCTTCTCCACGCGCGCGGGGAGGCAGGGCTCCACCGCCAGTCTGGAAGGGTTCCACATACAGGAACGGCCTAC  
TTCGCAGATGAGCCCACCGAGGCTCAGGCTCCGGGCGGATTCTGCGTGTACCCCTCGCTCCTTGGGGTCCGCTGGCCGGC  
CTGTGCCACCCGGACGCCCGGCTCACTGCCTCTGTCTCCCCCATCAGCGCAGCCCCGGACGCTATGGCCCACCCCTCCAG  
CTGGCCCCCTCGAGTAGGATGGTAGCACGTAACCAGGTGGCAGCCGACAATGCGATCTCCCCGGCATCAGAGCCCCGACGG  
CGGCCAGAGCCATCCTCGTCTCGTCTTCGTCTCGCCGGCGGGCCCCGGCGGCTCCCCGGCCCTGCCCGGTGGTCCCGGC  
CCCGGCTCCGGGCGACACTCACTTCCGCACCTTCCGCTCCCACTCTGATTACCGGCGCATCACGCGGACCAGCGCTCTCC  
TGGACGCCCTGCGGCTTCTACTGGGGACCCCTGAGCGTGCATGGGGCGCACGAACGGCTGCGTGCCGAGCCCGTGGGCACC  
TTCTTGGTGCGCGACAGTCGCCAGCGGAAGTGTCTTTCGCGCTCAGCGTGAAGATGGCTTCGGGCCCCACGAGCATTCG  
TGTGCACCTCCAGGCCGGCCGCTTCCACCTGGACGGCAGCCGCGAGACCTTCGACTGCCTCTTCGAGCTGCTGGAGCACT  
ACGTGGCGGCGCCGCGCCGCTGTTGGGGGCCCCACTGCGCCAGCGCCGCGTGGCGCCGCTGCAGGAGCTGTGTGCCAG  
CGCATCGTGGCCGCGTGGGTGCGGAGAACCTGGCACGCATCCCTCTTAACCCGGTACTCCGTGACTACCTGAGTTCCTT  
CCCCCTCCAGATCTGACCGGCTGCCGCGTGGCCGCGAGCATTAAGTGGGAGCGCCTTATTATTCTTATTATTAAATTATT  
ATTATTATTTCTGGAACACGTTGGGAGCCCTCCCCGCTAGGTTCGGAGGGAGTGGGTGTGGAGGGTGAGATGCCCTCCCACT  
TCTGGCTGGGACCTTATCCCGCTCTCGGGGGGCTCCCCCTCCTGGTGTCCCTCCCGGTCCCCCTGGTTGTAGCAGCT  
TGTGTCTGGGGCCAGGACCTGAACTCCACGCCTACCTCTCCATGTTTACATGTTCCAGTATCTTTGCACAAACCAGGGG  
TGGGGGAGGGTCTCTGGCTTCATTTTCTGCTGTGCAGAAATATTCTATTTTATATTTTACATCCAGTTTAGATAATAAA  
CTTTATTATGAAAGTTTTTTTTTTAAAGAAAAAAAAAAAAAAAAAAAAA

Fig. 30

Rat 25r DNA (coding 130-

GGCACGGCTCCCGGCCCCGGAGCATGCGCGACAGCAGCCCCGGAACCCCCAGCCGCGGCGCCCCGCGTCCCGCCGCCAGC  
GCAGCCCCGGACGCTATGGCCCACCCCTCCAGCTGGCCCCCTCGAGTAGGATGGTAGCACGTAACCAGGTGGCAGCCGACA  
ATGCGATCTCCCCGGCATCAGAGCCCCGACGGCGGCCAGAGCCATCCTCGTCCTCGTCTTCGTCTCGCCGGCGGCCCCG  
GCGCGTCCCCGGCCCTGCCCGGTGGTCCCCGGCCCCGGCTCCGGGCGACACTCACTTCCGCACCTTCCGCTCCCACTCTGA  
TTACCGGGCGATCACGCGGACCAGCGCTCTCCTGGACGCCTGCGGCTTCTACTGGGGACCCCTGAGCGTGTCATGGGGCGC  
ACGAACGGCTGCGTGCCGAGCCCGTGGGCACCTTCTTGGTGCGCGACAGTCGCCAGCGGAACCTGCTTCTTCGCGCTCAGC  
GTGAAGATGGCTTCGGGCCCCACGAGCATTCGTGTGCACTTCCAGGCCGGCCGCTTCCACCTGGACGGCAGCCGCGAGAC  
CTTCGACTGCCTCTTCGAGCTGCTGGAGCACTACGTGGCGGCGCCGCGCCGATGTTGGGGGCCCCACTGCGCCAGCGCC  
GCGTGCGGCGCGCTGCAGGAGCTGTGTGCCAGCGCATCGTGGCCGCGCGTGGGTGCGGAGAACCTGGCAGCATCCCTCTT  
AACCCGGTACTCCGTGACTACCTGAGTTCCTTCCCCTTCCAGATCTGACCGGCTGCCGCCGTGCCCGCAGCATTAAGTGG  
GAGCGCCTTATTATTTCTTATTATTAATTATTATTATTTTCTGGAAACCACGTGGGAGCCCTCCCCGCCTAGGTCCGAGG  
GAGTGGGTGTGGAGGGTGAGATGCCTCCCACTTCTGGCTGGAGACCTTATCCCGCCTCTCGGGGGGCTCCCTCCTGGT  
GCTCCCTCCCGGTCCCCCTGGTTGTAGCAGCTTGTGTCTGGGGCCAGGACCTGAACTCCACGCCCTACCTCTCCATGTTTA  
CATGTTCCAGTATCTTTGCACAAACCAGGGGTGGGGGAGGGTCTCTGGCTTCATTTTTCTGCTGTGCAGAAATATTTCTAT  
TTTATATTTTTACATCCAGTTTAGATAATAAACTTTATTATGAAAGTTTTTTTTTTTAAAAA

Fig. 31

## Rat 5p protein

MPSQMEHAMETMMLTFHRFAGEKNYLTKEDLRVLMEREFPGFLENQKDPLAVDKIMKDLDQCRDGKVGFSFLSLVAGLI  
IACNDYFVVHMKQKK

## Rat 5p DNA (coding: 52-339)

CTTCCAAAGACTGCAGCGCCTCAGGGCCCAGGTTTCAACAGATTCTTCAAAATGCCATCCCAAATGGAGCATGCCATGGA  
AACCATGATGCTTACATTTACAGGTTTGCAGGGGAAAAAACTACTTGACAAAGGAGGACCTGAGAGTGCTCATGGAAA  
GGGAGTTCCCTGGGTTTTTGGAAAATCAAAGGACCCTCTGGCTGTGGACAAAATAATGAAAGACCTGGACCAGTGCCGA  
GATGGAAAAGTGGGCTTCCAGAGCTTCTATCACTAGTGGCGGGGCTCATCATTGCATGCAATGACTATTTTGTAGTACA  
CATGAAGCAGAAGAAGTAGGCCAACTGGAGCCCTGGTACCCACACCTTGATGCGTCCTCTCCCATGGGGTCAACTGAGGA  
ATCTGCCCCACTGCTTCCTGTGAGCAGATCAGGACCCTTAGGAAATGTGCAATAACATCCAACCTCCAATTCGACAAGCA  
GAGAAAGAAAAGTTAATCCAATGACAGAGGAGCTTTCGAGTTTTATATTGTTTGCATCCGGTTGCCCTCAATAAAGAAAG  
TCTTTTTTTTTTAAGTTCGAAAAAAAAAAAAAAAAAAAAA

Fig. 32



## Rat 7q protein

MAYAYLFKYIIIGDTGVGKSCLLQFTDKRFQPVHDLTIGVEFGARMITIDGKQIKLQIWDTAGQESFRSITRSYYRGAA  
GALLVYDITRRDTFNHLTTWLEDARQHSNSNMVIMLIGNKSDLESRRREVKKEEGEAFAREHGLIFMETSAKTASNVEEAF  
INTAKEIYEKIQEGVFDINNEANGIKIGPQHAATNASHGGNQGQAGGGCC

## Rat 7q DNA (coding 1-639)

ATGGCGTACGCCTATCTCTTCAAGTACATCATCATCGGCGACACAGGTGTTGGTAAATCGTGCTTATTGCTACAGTTTAC  
AGACAAGAGGTTTCAGCCGGTGATGACCTCACAAATTGGTGTAGAGTTTGGTGCTCGAATGATAACCATTTGATGGGAAAC  
AGATAAAACTCCAGATCTGGGATACAGCAGGGCAGGAGTCCCTTCGTTCTATCACAAGGTCATATTACAGAGGTGCAGCG  
GGGGCTTTACTAGTGTATGATATTACAAGGAGAGACACGTTCAACCACTTGACAACCTGGTTAGAAGACGCCCCTCAGCA  
TTCCAATTCCAACATGGTCATCATGCTTATTGGAAATAAAAGTGACTTAGAATCTAGGAGAGAAGTGAAAAAGGAAGAAG  
GTGAAGCTTTGCACGAGAGCATGGACTTATCTTCATGGAACTTCTGCCAAGACTGCTTCTAATGTAGAGGAGGCATTT  
ATTAACACAGCAAAAGAAATTTATGAAAAATCCAAGAAGGGGTCTTTGACATTAATAATGAGGCAAACGGCATCAAAAT  
TGGCCCTCAGCATGCTGCTACCAATGCATCTCACGGAGGCAACCAAGGAGGGCAGCAGGCAGGGGGAGGCTGCTGCTGA

Fig. 33

## Rat 19r protein

MVLLKEYRVILPVSVD EYQVGQLYSVAEASKNETGGGEGVEVLVNEPYEKDDGEKGQYTHKIYHLQSKVPTFVRMLAPEG  
ALNIHEKAWNAYPYCRTVITNEYMKEDFLIKIETWHKPD LGTQENVH KLEPEAWKHVEAIYIDIADRSQVLSKDYKAEED  
PAKFKSIKTGRGPLGNWKQELVNQKDCPYMCAYKLVTVKFKW WGLQNKVENFIHKQEKRLFTNFHRQLFCWLDKWVDLT  
MDDIRRMEEETKRQLDEMROKDPVKGMTADD

## Rat 19r DNA (coding 1-816)

ATGGTGCTGCTCAAGGAATATCGGGTCATCCTGCCTGTGTCTGTAGATGAGTATCAAGTGGGGCAGCTGTACTCTGTGGC  
TGAAGCCAGTAAAAATGAACTGGTGGTGGGGAAGGTGTGGAGGTCTGGTGAACGAGCCCTACGAGAAGGATGATGGCG  
AGAAAGGCCAGTACACACACAAGATCTACCACTTACAGAGCAAAGTTCCACGTTTGTTCGAATGCTGGCCCCAGAAGGC  
GCCCTGAATATACATGAGAAAGCCTGGAATGCCTACCCCTTACTGCAGAACCGTTATTACAAATGAGTACATGAAGGAAGA  
CTTTCTCATTAAAAATTGAAACCTGGCACAAGCCAGACCTTGGCACCAGGAGAATGTGCATAAACTGGAGCCTGAGGCAT  
GGAAACATGTGGAAGCTATATATATAGACATCGCTGATCGAAGCCAAGTACTTAGCAAGGATTACAAGGCAGAGGAAGAC  
CCAGCAAAATTTAAATCTATCAAAACAGGACGAGGACCATTGGGCCCCGAATTGGAAGCAAGAACTTGTCAATCAGAAGGA  
CTGCCCATATATGTGTGCATACAACTGGTTACTGTCAAGTTCAGTGGTGGGGCTTGCAGAACAAAGTGGAAAACTTTA  
TACATAAGCAAGAGAAGCGTCTGTTTACAACTTTCAAGGCAGCTGTTCTGTTGGCTTGATAAATGGGTTGATCTGACT  
ATGGATGACATTCGGAGGATGGAAGAAGAGACGAAGAGACAGCTGGATGAGATGAGACAAAAGGACCCCGTGAAAGGAAT  
GACAGCAGATGACTAG

Fig. 34

Monkey KChIP4c (jlkxa053c02) DNA sequence (CD: 122-811)

CGCTCCTCCTCCTCCCTTTCTCTAGCAGTAGCCTTCTTAATGTAGTTTAATGGCTTTACAAAGAAAGCCAGGCAGAGGAG  
CACTTCTCAGTGGCTGTGGTCGGACCATGACCTAGCTGACCATGAACTTGAAGGGCTTGAAATGATAGCAGTTCTGATC  
GTCATTGTGCTTTTTGTTAAATTATTGGAACAGTTTGGGCTGATTGAAGCAGGTTTAGAAGACAGCGTGGAAGATGAACT  
GGAGATGGCCACTGTCTAGGCATCGGCCGTAGGCCCTTGAGCTTCTGGAAGCCAGAGCAAATTTACCAAGAAAGAGCTTC  
AGATCCTTTACAGAGGATTTAAGAACGAATGCCCCAGTGGTGTGTTAATGAAGAAACCTTCAAAGAGATTTACTCGCAG  
TTCCTTCCACAGGGAGACTCTACAACATATGCACATTTTCTGTTCAATGCGTTTGATACGGACCACAATGGAGCTGTGAG  
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CAGGCTGTTTTCCATTGTATCATCAAGTGGAAGTTCAAGACGGCATCAAACAAAAAAGGATGTTTACAGACATATGCAA  
AGGGTCAGGATATCTATCCTCCAGTATATGTTAATGCTTAATAACAAGTAATCCTAACAGCATTAAAGGCCAAATCTGTC  
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ACTAGGAACAAACAAACAGCAAGCAAAATTCACCTTTGAAAGCACCAGTGGTTCCATTACATTGACAACTACTACCAAGAT  
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CACAAAGACCAAGAGGCTACAGAAGGAGGAAATTTGCAACTGTCTTTGCAACAATAAATCAGGTATCTATTCTGGTGTAG  
AGATAGGATGTTGAAAGCTGCCCTGCTATCACCAGTGTAGAAATTAAGAGTAGTACAATACATGTACACTGAAATTTGCC  
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TCTAAAAA

Monkey KChIP4c protein sequence

MNLEGLEMI AVLIVIVLFVKLLEQFGLIEAGLEDSVEDELEMATVHRPEALELLEAQSKFTKKELQILYRGFKNECP  
V VNEETFKRIYSQFFPQGDSTTYAHFLFNAFDTHNGAVSFEDFIKGLSILLRGTVQEKLNWAFNLYDINKDGYITKEEM  
LDIMKAIYDMMGKCTYPVLKEDAPRQHVETFFQKMDKNKDGVTIDEFIESCQKDNIMRSMQLFENVI.

Fig. 35

Monkey KChIP4d (j1kx015b10) DNA sequence (CD:64-816)

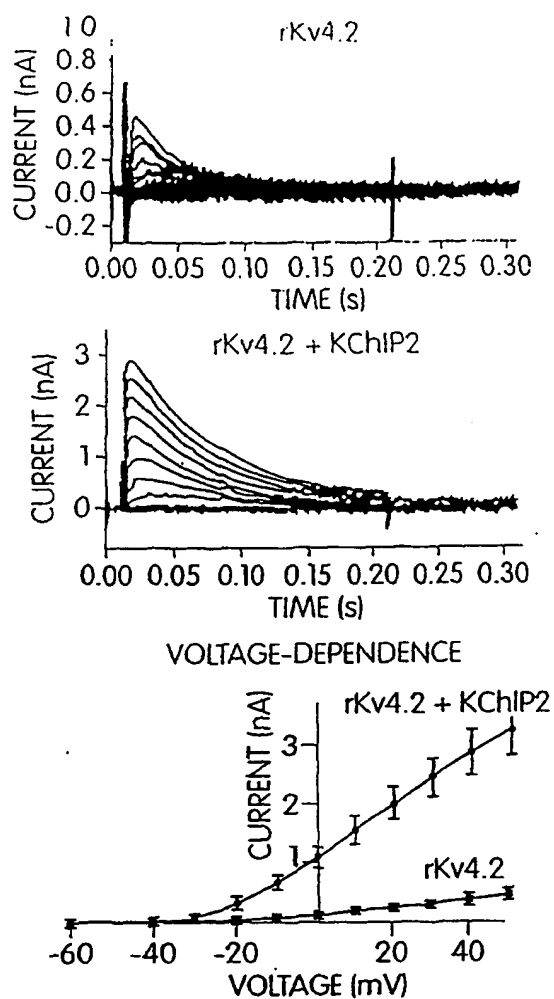
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Monkey KChIP4d protein sequence

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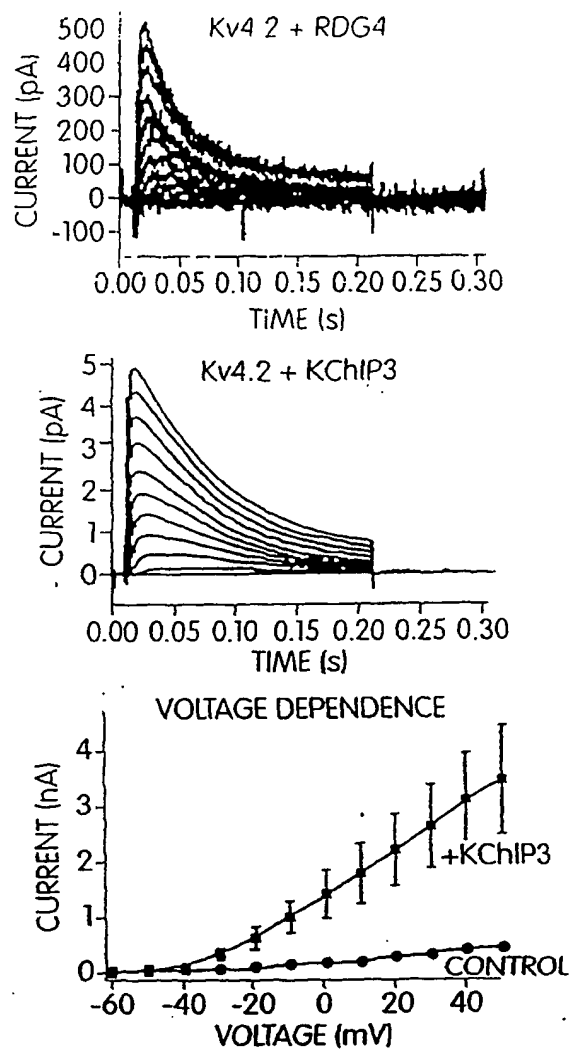
Fig. 36





CURRENT PARAMETER	CHO	
	rKv4.2	rKv4.2 + KChIP2
PEAK CURRENT (nA/cell, at 50 mV)	0.51 ±0.098	3.3 ±0.45
PEAK CURRENT DENSITY (pA/pF, at 50 mV)	18.6 ±2.8	196.6 ±26.6
INACTIVATION TIME CONSTANT (ms, at 50 mV)	28.47 ±3.5	95.14 ±8.3
RECOVERY FROM INACTIVATION TIME CONSTANT (ms, at -80 mV)	257.9	49.5
ACTIVATION $V_{1/2}$ (mV)	20.5	-2.2
STEADY-STATE INACTIVATION $V_{1/2}$ (mV)	-47.1	-45.7

Fig. 38



CURRENT PARAMETER	CHO	
	rKv4.2 +RBG4	rKv4.2 +KChIP3
PEAK CURRENT (nA/cell, at 50 mV)	0.46 ±0.084	3.5 ±0.99
PEAK CURRENT DENSITY (pA/pF, at 50 mV)	29.7 ±11.2	161.7 ±21.8
INACTIVATION TIME CONSTANT (ms, at 50 mV)	29.5 ±9.5	67.2 ±14.1
RECOVERY FROM INACTIVATION TIME CONSTANT (ms, at -80 mV)	435.9	130.8
ACTIVATION $V_{1/2}$ (mV)	4.1	6.1

Fig. 39

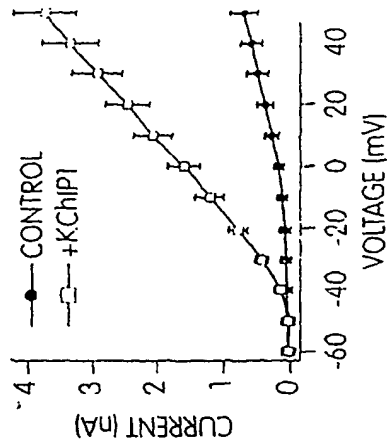


Fig. 40C

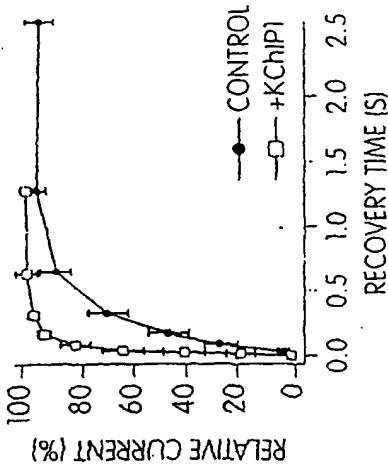


Fig. 40F

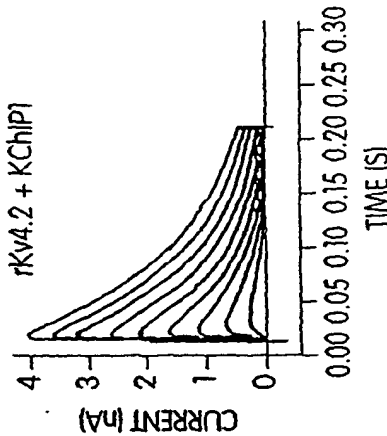


Fig. 40B

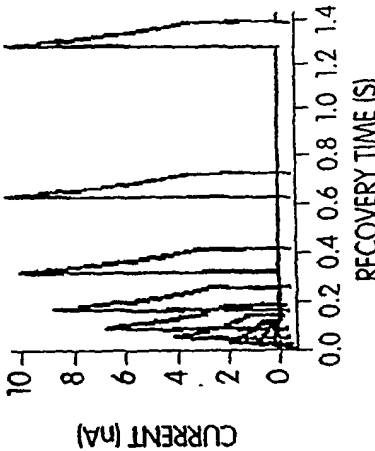


Fig. 40E

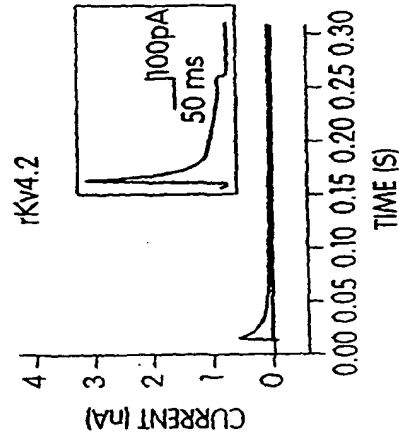


Fig. 40A

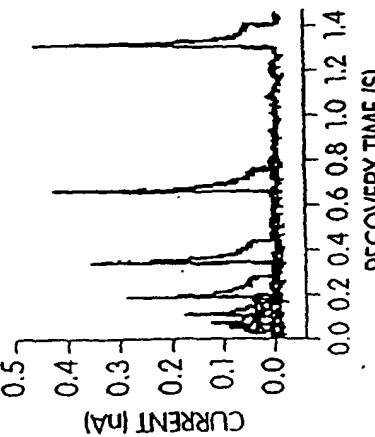


Fig. 40D



[illegible]

Fig. 41

NIKALI ET AL.

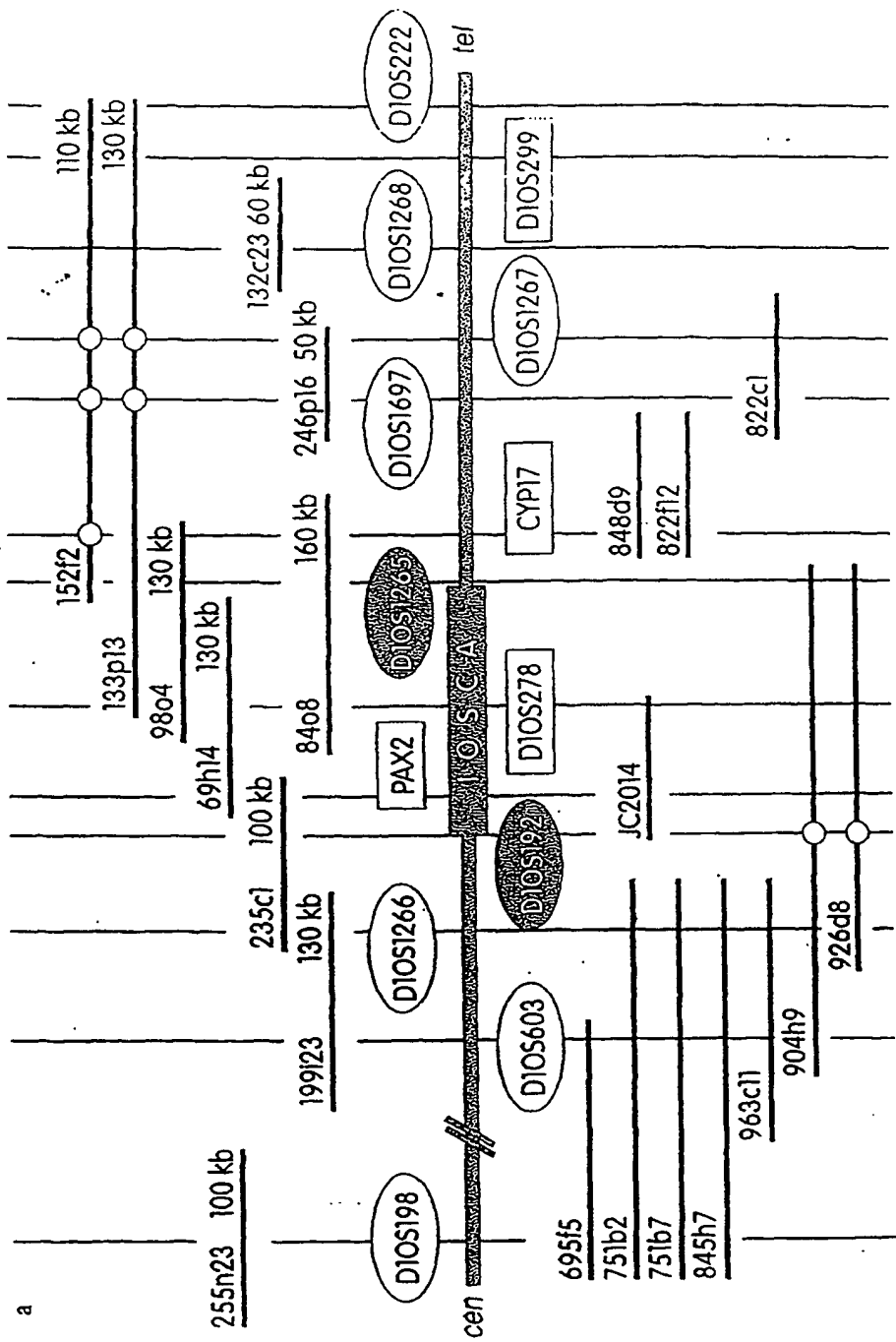


Fig. 42

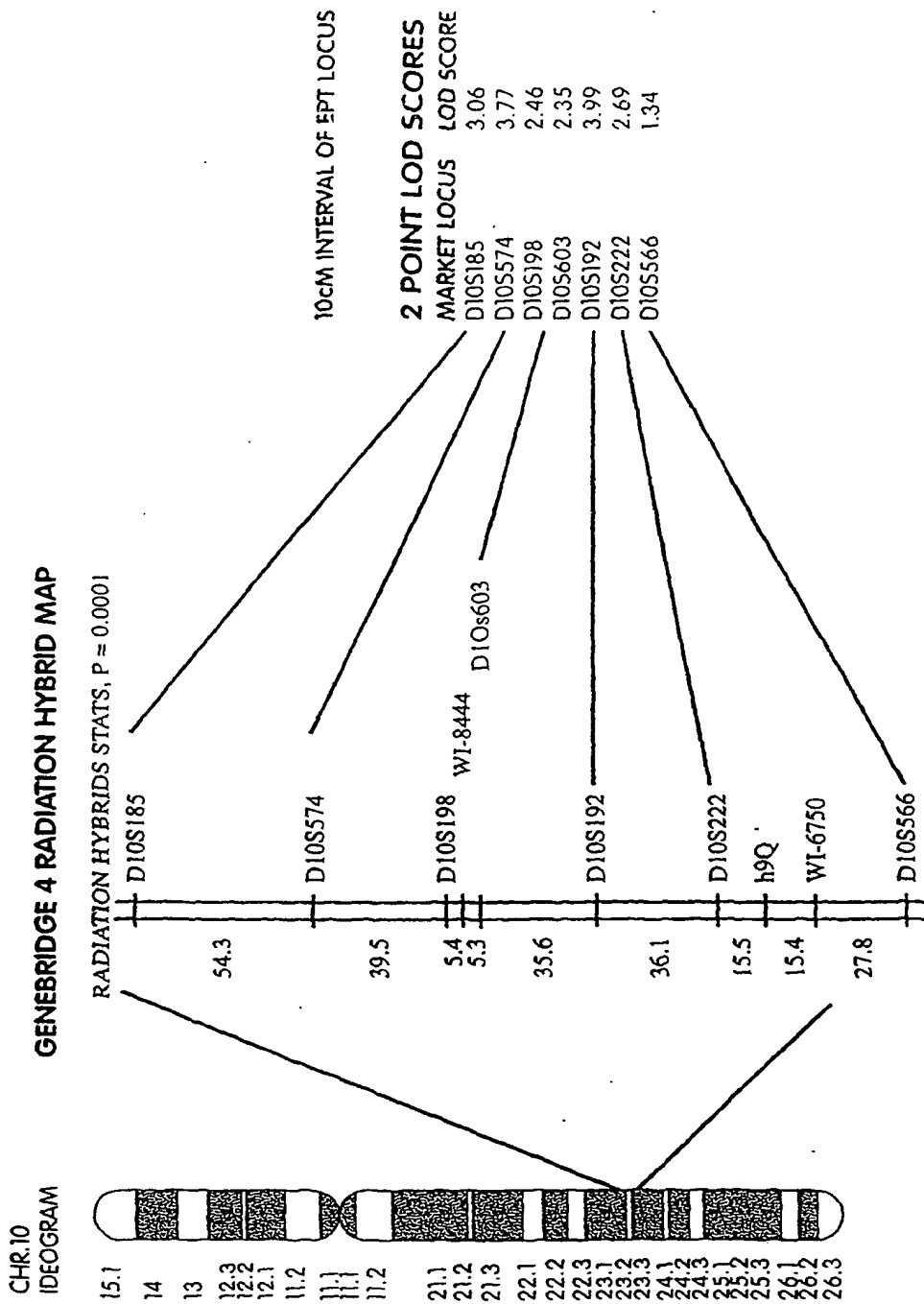


Fig. 43

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FIGURE 44

KChIP1N (1vn) DNA sequence (CD: 353-1051. Alternation of lower and upper cases indicates individual exons.

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 TATTAAGCATGACATTGTCTGATGACCCAAACTGCCCCG

#### KChIP1N (1vn) protein sequence

MWAEGESEGLQTLGIVVVVCSLLKLLHYLGLIDLSDDKIEDELEMTMVCHRPEGLEQLEAQTNFTKRELQVLYRGFKNEC  
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 EEMMDIVKAIYDMMGKYTPVLKEDTFRQHVDFVQKMDKNKDGIVTLDEFLESCQEDDNIMRSLQLFQNVN

FIGURE 45

10 20 30

1 M L T Q G E S E G L O T L G I V V V I C S S L F L L H Y L G L I D L S D

1 M W A E G E S E G L Q T L G I V V V C S S L K L L H Y L G L I D L S D

v

rkchaplN  
NKDPPIN  
N.pap

Decoration 'Decoration #1': Shaded residues are the ones that differ from  
rkchaplN N.pap.

FIGURE 46

[illegible]

FIGURE 47

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 gctcgtgtgtcctcgtctgtttgtcaaggctctggagcaggttggcctctttagcctgtctccttgaaggtaatccag  
 gtgtcctggcatgtctgagctgtgtgtgtgggagctgcccacagcactagggaggggtgtttgtctgtcttcatga  
 ctggggcagggatgagcaggtgagtgatggcacagggatctgggaggtgagtaggggtcttgggggatacaacatctgg  
 gagggtgaagaggagggcctcaaggagtagatgaggaggagggaggaatctgggggaccaaggtgtcaggaggtgagta  
 ggggtgagtgaggtgggtgtgtgagatgaggaggtgaggggattctgggcgataaccaggaggggtgtgagat  
 aggggaaggaggtgtctagagtagaggagggaggttctaggagcctgagataacaacggatttctgggggtccttctgg  
 agtcaggggcccaccaggtgctgtgcatctctccacacctccatctgtgcagtgtagcttatggggtgtgtggcat  
 agtgtgtgagagacacaggggtgtgactttgtgttctgtatgtgagagtagtaaaagtgtgtaactgtgagcctgtgtat  
 gggacagggatgtgactgtatgtgtgagagtgacagggcatgtggcttgtgagaaggtggatgtggccagggcctgagtg  
 gcacagtatgtgtgaggtgtgggtgtgggtgtgggtgtgggtgtgttccagaattctgtgaggtatataccagctcc  
 ataccaagggcttgggagggaggtgggttctggaggggaagagtggaacattgtgaaggagacattttagccagctatg  
 attggggatgaggagtgagtagatcagagcaggtgttcccaagtccttgcctctcagagctcctatcaggatccaa  
 gtgcagagtgatctccagggcctagacattgggagtggaatcaactccctcccgagccaccccaagtcagtaggg  
 agagtaaatattgggggtgagtgaggagcactctgctcccccttgggagtttgacctctccctcctctccagggctag

FIGURE 47 (cont'd)





TGTCTCAAAAAGgtacagcTccclTgacctctacattaccctgacctgqactcraggcctgatttagtaatgcagggaagagclt  
 ctctgggaagaataccacctTcccacctcacccccataatttcaaiacclacclctctggggagcctTcccacctTcccac  
 ctccaggctctctclggcactclccctctctctctgctcttgaattgcccgTctgtgactcagtgctccctctcaactclctct  
 qtaagactcctctctctctctclctclctctcaatctgctctgctcctacatTcaltgccacagGATGAGAACATCATGAGGTCCAT  
 GCAGCTCTTTGACAATGTCATCTAGCCCCCAGGAGAGGGGGTCAGTGTTTCTGGGGGGACCATGCTCTAACCTTAGTCC  
 AGGCGGACCTCACCTTCTCTTCCCAGGTCATCTCATCTACGCTCCCTGGGGGCTGGAGGGATCCAAGAGCTTGGG  
 GATTTCAGTAGTCCAGATCTCTGGAGCTGAAGGGGGCAGAGAGTGGGCAGAGTGCATCTGGGGGGTGTTCCCAACTCCCA  
 CAGACTCTACCCCCCTTCTGCTGTACACCCAGTGTTGAGAGTGCCTCTCTGTAGGAATTTGAGCGGTTCCCCACTCTCT  
 ACCCTACTCTAGAAACACACTAGACAGATGTCTCTGCTATGGTGCTTCCCCATCCCTGACCTCATAAACATTTCCCC  
 TAAGACTCCCTCTCAGAGAGAATGCTCCATTCTTGGCACTGGCTGGCTTCTCAGACAGCCATTGAGAGCCCTGTGGGA  
 GGGGGACAGAAGATGTATAGGAGAAATCTTGGGCTGAGTCAATGGATAGGCTCTAGGAGGTGGCTGGGGTTGAGAATAG  
 AAGGGCTGACAGATTATGATTGCTCAGGCATACAGGTTATAGCTCCAAGTTCCACAGTCTGCTCCACAGGCCAATC  
 AAAATATAAGATTTCAGGCTTTGAGGAAGACCTTGTCTCCTTAGAAATGCCCCAGAAATTTCCACACCCTCTCGGTAT  
 CCATGGAGAGCCTGGGGCCAGATATCTGGCTCATCTCTGGCATTGCTTCTCTCCTTCTCTCTGCTGTGTTGGTGGTG  
 GTTGTGTGGTGGGGAAATGTGGATGGGGGATGTCTGGCTGATGCCTGCCAAATTTTCATCCACCTCTCTTGTCTATGCTC  
 UCTGTTTGTAGGGGCTATGACTTGAGTTTGTGTTCCCATGTTCTCTATAGACTTGGGACCTCTGAACTTGGGGCTAT  
 CACTCCCCACAGTGGATGCTTTAGAAGGAGAGGGAAGGAGGAGGAGGCATGAGCATGACTGTAACCCAGTGTGGGGCATT  
 CACTAGAATCTTCAATCAACCTGGGCTCTCCCCACCCACCCACAGATAACCTCCTCAGTTCCCTAGGGTCTCTTCTTGCT  
 TGACTCAATCTACCCAGAGATGCCCTTAGCACACCTAGAGGGCAGGGACCATAGGACCCAGGTTCCAACCCCATTTGTCA  
 GCACCCACCCATGCGGCCACCCCTTAGCACACCTGCTGCTCCACTTAGCTTACCCTCCAGTTGGCCAGAATCTGAGG  
 GGAGAGCCCCCAGAGAGCCCCCTTCCCCATCAGAAGACTGTGTGACTGCTTTGCATTTTGGGCTCTTCTATATATTTGTGA  
 AAGTAAAGAAATATACAGATCTAATAAAACACATGGCTATGC

**FIGURE 47 (cont'd)**

## Rat kchip21 DNA (CD: 1-813)

ATGCGGGGCCAAGGCAGAAAGGAGAGTTTGTCCGAATCCCGAGATCTGGACGGCTCCTATGACCAGCTTACGGGCCACCC  
TCCAGGGCCCCAGTAAAAAGCCCTGAAGCAGCGTTTCCTCAAGCTGCTGCCGTGCTGCGGGCCCCAAGCCCTGCCCTCAG  
TCAGTGAAACATTAGCTGCCCCAGCCTCCCTCCGCCCCACAGACCCGCCCCGCTGGACCCAGACAGCGTAGAGGATGAG  
TTTGAATTATCCACGGTGTGTACCCGACCTGAGGGCCTGGAACAACCTCCAGGAACAGACCAAGTTCACACGCAGAGAGCT  
GCAGGTCCTGTACCGAGGCTTCAAGAACGAATGCCCCAGTGGGATTGTCAACGAGGAGAACTTCAAGCAGATTTATTCTC  
AGTTCTTTCCCAAGGAGACTCCAGCAACTATGCTACTTTTCTCTTCAATGCCTTTGACACCAACCACGATGGCTCTGTC  
AGTTTTGAGGACTTTGTGGCTGGTTTGTCCGTGATTCTTCGGGGGACCATAGATGATAGACTGAGCTGGGCTTCAACTT  
ATATGACCTCAACAAGGACGGCTGTATCACAAAGGAGGAAATGCTTGACATTATGAAGTCCATCTATGACATGATGGGCA  
AGTACACATACCCTGCCCTCCGGGAGGAGGCCCCAAGAGAACACGTGGAGAGCTTCTCCAGAAGATGGACAGGAACAAG  
GACGGCGTGGTGACCATCGAGGAATTCATCGAGTCTTGTCAACAGGACGAGAACATCATGAGGTCCATGCAGCTCTTTGA  
TAATGTCATCTAGCTCCCCAGGAGAGGGGTTAGTGTGTCTTAGGGTGACAGGCTGTAGTCTAGTCCAGACGAACCTA  
ACCCTCTCTCTCCAGGCCTGTCTCATCTTACCTGTACCCTGGGGGCTGTAGGGATTCAATATCCTGGGGCTTCAGTAGT  
CCAGATCCCTGAGCTAAGTCACAAAAGTAGGCAAGAGTAGGCAAGCTAAATCTGGGGGCTTCCCAACCCCCGACAGCTCT  
CAGCCCTTCTCAACTGATACCTAGTGTGTGAGGACACCCCTGGTGTAGGGACCAAGTGGTTCTCCACCTTCTAGTCCCACT  
CTAGAAACCACATTAGACAGAAGGTCTCCTGCTATGGTGCTTTCCCATCCCTAATCTCTTAGATTTTCTCAAGACTCC  
CTTCTCAGAGAACACGCTCTGTCCATGTCCCCAGCTGGGGACATGGACAGAGCGTGTCTCTAGTTCTAGATCGCGAGCG  
GCCGC

## Rat kchip21 protein

MRGQGRKESLSERDLGSDYDQLTGHPGPSKKALKQRFLKLLPCCGPQALPSVSETLAAPASLRPHRPRPLDPDSVEDE  
FELSTVCHRPEGLEQLQEQTKEFTRRELQVLYRGFKNECPSGIVNEENFKQIYSQFFPQGDSSNYATFLNADFDTNHDGSV  
SFEDFVAGLSVILRGTTIDRLSWAFNLYDLNKDGCITKEEMLDIMKSIYDMMGKYTYPALREEAPREHVESFFQKMDRKN  
DGVVTIEEFIESCQDENIMRSMQLFDNVI

FIGURE 48

human kchip2N DNA (CD:1-678; 1a is added based on genomic sequence)

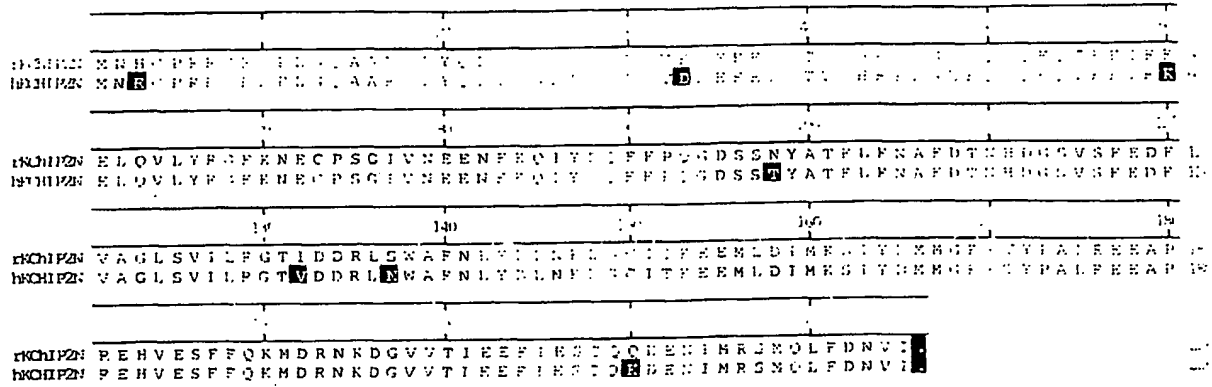
aTGAACCGATGCCCCCGCAGGTGCCGGAGCCCGCTGGGGCAGGCAGCGGATCCCTCTACCACTGGTGAAGTGGGTGCT  
GTCCCCAGACAGCGTGGACGATGAATTTGAATTGTCCACCGTGTGTACCGGCTGAGGGTCTGGAGCAGCTGCAGGAGC  
AAACCAAATTCACGCGCAAGGAGTTGCAGGTCTGTACCGGGCTTCAAGAACGAATGTCCAGCGGAATTGTCAATGAG  
GAGAACTTCAAGCAGATTTACTCCAGTTCTTCTCAAGGAGACTCCAGCACCTATGCCACTTTTCTCTTCAATGCCTT  
TGACACCAACCATGATGGCTCGGTCAATTTGAGGACTTTGTGGCTGGTTTGTCCGTGATCTTCCGGGAAGTGTAGATG  
ACAGGCTTAATTTGGGCTTCAACCTGTATGACCTTAACAAGGACGGCTGCATCACCAGGAGGAAATGCTTGACATCATG  
AAGTCCATCTATGACATGATGGGCAAGTACAGTACCCTGCACTCCGGGAGGAGGCCCAAGGGAACACGTGGAGAGCTT  
CTTCCAGAAGATGGACAGAAACAAGGATGGTGTGGTGACCATTTAGGGAATTCATTGAGTCTTGTCAAAGGATGAGAA  
TCATGAGTCCATGCACTCTTTGACAATGTCATCTAGCCCCAGGAGAGGGGTCAGTGTTCCTGAGGGACCATGCT  
CTAACCTTAGTCCAGGCGGACCTCACCTTCTCTTCCAGGTCTATCCTCATCTACGCTCCCTGGGGGCTGGAGGGAT  
CCAAGAGCTTGGGGATTCACTAGTCCAGATCTCTGGAGCTGAAGGGGCCAGAGAGTGGGCAGAGTGCATCTCGGGGGGTG  
TTCCCAACTCCCTCCAGCTCTCACCCCTTCTGCTGACACCCAGTCTTGAGAGTGCCTCTCTGTATGAATTTAGCGG  
TTCCCCACTCTCTACCCCTACTCTAGAAACACACTAGACAGATGTCTCTGCTATGGTGCTTCCCCATCCCTGACCTCA  
TAAACATTTCCCTAAGACTCCCTCTCAGAGAGAATGCTCCATTCTGGCACTGGCTGGCTTCTCAGACCAGCCATTGA  
GAGCCCTGTGGGAGGGGGACAAGAATGTATAGGGAGAAATCTTGGGCTGAGTCAATGGATAGGTCTTAGGAGGTGGCTG  
GGGTTGAGAATAGAAGGGCCTGGACAGATTATGATTGCTCAGGCATACCAGGTTATAGTCCAAGTTCACAGGTCTGCT  
ACCACAGGCCATCAAAATATAAGTTTCCAGGCTTTGCAGAAGACCTTGTCTCCTTAGAAATGCCCCAGAAATTTCCACA  
CCCTCTCGGTATCCATGGAGAGCCTGGGGCCAGATATCTGGCTCATCTCTGGCATTGCTTCTCTCTCTCTCTCTGCA  
TGTGTTGGTGGTGGTGTGGTGGGGGAATGTGGATGGGGGATGTCTGGCTGATGCCTGCCAAAATTTATCCCACCTC  
CTTGCTTATCGTCCCTGTTTTGAGGGCTATGACTTGAGTTTTTGTTCCTATGTTCTCTATAGACTTGGGACCTTCTCTGA  
ACTTGGGGCTATCACTCCCCACAGTGGATGCCTTAAAGGGAGAGGGAAGGAGGGAGGCAGGCATAGCATCTGAACCCA  
GTGTGGGGCATTCACTAGAATCTTCAATCAACCTGGGCTCTCCCCACCCACCCAGATAACCTCCTCAGTTCCCTAGG  
GTCTCTTCTTGCTTGACTCAATCTACCCAGAGATGCCCCCTTAGCACACCTAGAGGGCAGGGACCATAGGACCCAGGTTCC  
AACCCCATTTGTCAGCACCCAGCCATGCGGCCAACCCTTAGCACACCTGCTCGTCCCATTTAGCTTACCTCCCAGTTGG  
CCAGAATCTGAGGGGAGAGCCCCCAGAGAGCCCCCTTCCCATCAGAAGACTGTTGACTGCTTTGCATTTTGGGCTCTTC  
TATATATTTTGTAAAGTAAGAAATATACCAGATCTAATAAAACACAATGGCTATGCACAGAAAAAAAAAAAAAAAAA

human kchip2N protein

MNRCPRRCRSPGQAARSLYQLVTGSLSPDSVDDEFELSTVCHRPEGLEQLQEQTKEFTRKELQVLYRGFKNECPSGIVNE  
ENFKQIYSQFFPQGDSSYATFLFNAFDTNHDSVVSFEDFVAGLSVILRGTVDDRLNWFNLYDLNKGDCITKEMLDIM  
KSIYDMMGKYTPALREEAPREHVESFFQKMDRNDKGVVTIEEFIESQKDENIMRSMQLFDNVI

FIGURE 49

Human and rat kch1p2n proteins are highly conserved (96.5% identity).



Decoration: "Decoration" (with solid bright, really) indicates the differ from hKCH1P2N.

FIGURE 50

**FIGURE 51**







Mouse kchip4c(kchip4N2) cDNA (CD:56-745)

GCCAGGGTGAGGAGCGCTTCTCAGTGGCTGTGGCTGGACCATGACctagCTGACCATGAACTTGGAGGGGCTTGAAATGA  
TAGCAGTTCTGATCGTCATTGTGCTTTTGTAAATTATTGGAAACAGTTTGGGCTGATTGAAGCAGGTTTAGAAGACAGC  
GTGGAAGATGAGCTGGAGATGGCTACTGTGAGGCATCGGCCTGAAGCCCTGGAGCTGCTGGAGGCCAGAGCAAATTCAC  
CAAGAAAGAGCTTCAGATTCTTTACAGAGGATTTAAGAATGAATGCCCCAGTGGTGTGTTAATGAAGAACTTTCAAGG  
AGATTTACTCACAGTTCTTTCCACAGGGAGACTCCACCACATATGCACATTTTCTCTCAATGCATTGACACGGACCAC  
AATGGAGCTGTGAGCTTTGAGGATTTTCATCAAAGGTCTTTCCATTTTGCTTCGAGGGACAGTACAAGAAAACTCAACTG  
GGCATTTAATTTGTATGACATAAACAAAGATGGCTACATCACTAAAGAAGAAATGCTGGACATAATGAAAGCAATCTACG  
ACATGATGGGGAAATGCACATACCCGGTCTCAAGGAAGATGCTCCCGACAGCATGTGGAGACGTTCTTCAGAAGATG  
GACAAAAATAAGATGGTGTGCTTACCATAGATGAGTTTCATTGAAAGTTGCCAAAAAGATGAAACATAATGCGCTCCAT  
GCAGCTCTTTGAAAATGTGATCTAGAATGTGAGCACCTCTCGACCGAAGAGGCAAATGTGAACGACTACACACAAGTTG  
AAGCCCCCACTTGTAGCATAGATAGCTCAGCTTTACACTGAGGCAGATTATGCAACAGCTTTGTTTTAATAAAAAGCAA  
CCCACCACCACCACCAAAATTAAGTTTCCAGTTACAAATCTGCATCCATGTCAACGGGGTCAATGAAATGTGCTAACTTA  
TTTCATACTCAAAGGCACAGAATCTGGAATAGCTTTGATCCTTAGCCACGTTATTATTGAGGTTTTTACAGTTTCAGTGA  
TTTTAAACACCAGTGGGTTTTCTACTTGTGTTGTATGTATTAGCCCTGGGTTTTAAATGGTTTTCTAAATACTTACA  
TCTGCATTTAACTTCCAGAAAGTCAATGAACTTTTATTAAATTCGACTCATGTAACACTGAAAAATGAAACAAAGATTAC  
TACAATTTAAATAGACCAAAAAACACAGTCCCAATTTCTATGGCTTCTCCACCTGCTGTTAAAGATATTAAATGTATTGGC  
ATTTTTTTAAAGGACACTTAAAAAATTAGTTTTATTATCAGATGTTAGCATATACCTAATAAAATTATTTTAGTATTTGT  
TAATTTTCCATACTCAAGCCAAGGCTCTATATAATCCATGAACTTTGGACCTGTTCAATCTTACATGTAGACTGTTTTG  
TATTGTGTTATGAAGTAGAAATTCAAAGTGTCAAACAAACCAAGGATGTTTACAGACTTGCCAAGGGTCCGGATGTCTGT  
CCTGCAATGCCTAGTGACGCTTATTAACAAGTAACCCTAACAGCAGTAAAGGGCAGTTCTTGCCACCCTCCAAGCCCCCTT  
AATGTTTTTACAGCATGTTTATCATACATAAGCCATTCAAGGAACAGAGAATCCTTGACGCCCCAAGCCTACTAGGAATA  
ATGATCAAGTAACATACTCTTTGAGAACACCCGTGATTCTATAGTATTGGAAATTATACACAAGAATGTATAGAAAATGA  
CTGCAAACTGACGGTTCATCTGAAATGCATTATGATTTAGCACATCATATAGCTCAAAGGATTCATAGTCCTTTTCAGT  
GGTCTTAAGCCAAAATGTAGAGTTGCCACAACAGTACTATAGAGATACACATCTTCCCTGTTGCGCAGAAATACAAGAA  
CCAAGAGGATACAGGAGGAGAAAATTTACGACTGTCTGCAACAATAAATCAGGTATCTATTCTGGTGTAGAGATAGGATG  
TTGAAAGCTGCCCTGCTATCACCAGTGTAGGAATTAAGAGTAGTACAGTACATGTACAGAAATCTGCCATCGCGTGTG  
TGTAAGCTCAATGTGCACATTTGTATCTCAAACCGGAAAAATAAAGCAAATAAAGTGTTTATTACTCTAAAAA  
AAAAAAAAAAAAAAAAAAAA

Mouse kchip4c(kchip4N2) protein

MNLEGLEMI AVLIVIVLFVKLLEQFGLIEAGLEDSVEDELEMATVRHRPEALELLEAQSKFTKKELQILYRGFKNECPSG  
VVNEETFKEIYSQFFPQGDSTTYAHFLFNAFDTDHNGAVSFEDFIKGLSILLRGTVQEKLNWAFNLYDINKDGYITKEEM  
LDIMKAIYDMMGKCTYPVLKEDAPRQHVETFFQKMDKNKDGVTIDEFIESCQKDENIMRSMQLFENV I

FIGURE 54

Rat kchip4 cDNA (CD: 1-597, partial)

TTAGAAGACAGTGTGGAAGATGAACTGGAGATGGCCACTGT CAGGCACCGGCCTGAAGCCCTGGAGCTGCTGGAGGCCCA  
GAGCAAATTCACCAAGAAAGAGCTTCAGATCCTTTACAGAGGATTTAAGAATGAATGCCCCAGTGGTGTGTTAATGAAG  
AAACCTTCAAGGAGATTTACTCGCAGTTCCTCCACAGGGAGACTCCACCACATATGCACATTTTCTCTCAATGCATTTC  
GACACGGACCACAATGGAGCTGTGAGCTTTGAGGATTTTCATCAAAGGTCTTTCCATTTTGCTTCGAGGGACAGTACAAGA  
AAACTGAACTGGGCATTTAATTTGTATGACATAAACAAGATGGCTACATCACTAAAGAGGAAATGCTGGACATAATGA  
AAGCAATTTACGACATGATGGGGAATGCACATACCCTGTCTCAAGGAAGATGCTCCCCGACAGCACGTGGAGACATTT  
TTCAGAGATGGACAAGAATAAAGATGGTGTGTTACCATAGACGAGTTCATTGAAAGTTGCCAAAAAGATGAAAACAT  
AATGCGCTCCATGCAGCTCTTTGAAAATGTGATCTAGactgtcgggtgccttgaccggaggcaaatgtggacgactacaca  
cgagttgaagccaccatttctagcatagattgctcagctttacactgaggcatattatgcaaacagcttggtttaatat  
aaagacccccgcgccc aaatttaagttttccagttacaaatccgcatccacgtcactggggtcccgaatgtgctcactt  
atctcactctctgagaacactcaaeaggcacagaatctggaacagcttggatcctcagccacgtgttaacgggggctttta  
cagatgagtgattttaaaacaccagtggtTTTCTACTTGTGTTGATTTCAGCCCTGGATTTAAGTGGTTTTCTAAAAAT  
ATTTACATCTGCATTTAACTTCCAGAAAGCCAATGACCTTTTCATTTAACTCAATTCATGTAATACTGAAAAAAGGAACA  
AAGATTATTACAATTAAGAACCAAAACACAGTCCCGATTTCTATAGCTTCTCCACCTGCTGTTAAAGACAGTCATG  
TATTTGGCTTTTTTTTTTTTTTTTAAAAAGAACACTTAAAAAATTAGTTTATTATCAGATGTTAGCATATACCTAATAAAA  
TTATTTTAGTATTTGTAAATTTCCATATTCAGCCAAGGCTCTATATAATCCATGTAACCTTGGACCTGTTCAATCTTA  
CATGTAGACTGTTTTGTATTGTGTTCTGAAGTAGAAGTTCAAAGTGTCAAACAAACCAAGGATGTTTACAGACTTGCAAA  
GGGTCCAGATGTCTGTCTGCAATGCCTAGTGACGCTTATTAACCAGTAACCTGAAGAGCAGTAACCTGGCAATTCAGCC  
ACCACCCCTCCCCAAGCCCCTTCATGTTCTCAGCATGTTTATCACACACAAGCCATTACAGGACAGAGAATCCTTGAC  
TGCCCCAAGCCTACTAGGAATAAAGATCAAGCAAAATCTTCTTTGAAAACACCAGTGATTCTATCATATTGGAAATATA  
CATAAGAGTGATAGAAAACGAATGTAGACATTGGACAGTTCATCCGAATTGCATTATGATTAGCACATCATGTAGTTC  
AAAGGATTCACATTCCTTTCCGTGATCTTAAGCCAAAACGTAGAAATTGCCACAACAGTACTAGATATACACACATTCCC  
TGTTTCGTGGAAATCCAAGAACCAAGAGGATACGGGAAGAGAAAATTTGCGACTGTCTGCAACAATAAATCAGGTATCTA  
TTCTGGTGTAGAGATAGGATGTTGAGAGCCGCCCTGCTATCACCAGTGTAGGAATTAAGAGTAGTACAGTACATGTACAG  
AAATCTGCCATCGCGTGTGTTGTGTAAACTCAATGTGCACATTTTGTATCTCAAAAAGGAAAAATAAGCAAAATAAAGTG  
TTAAAAA

Rat kchip4 protein (partial)

LEDVSEDELEMATVRHRPEALELLEAQSKFTKKELQILYRGFKNECPSGVVNEETFKELIYSQFFPQGDSTTYAHFLFNAF  
DTDHNGAVSFEDFIKGLSILLRGTVQEKLNWAFNLYDINKDGYITKEEMLDIMKAIYDMMGKCTYPVLKEDAPRQHVET  
FQKMDKNKDGVTIDEFIESQKDENIMRSMQLFENV

FIGURE 55

human kchip4N1S cDNA (CD: 319-885)

GAGAGGTCCGTGCGCTGTGGTAGCAGGGGGGAAGCCCCGCCAGCCAAATGCCAGGATCAGCATGAGAAGCTGGACTTTAG  
CCCAGGTCTGTCTCACCCTGGGGGGCCGCGGCTTTGTCAGGGTGCATCTGCCAGGAGCTGCTCACTTTTTCCCTTGCA  
AGTCTTTGTTCCAAGCTGACGTTGCTACGATTCTGTAATTAATCCCTCCACTCCAAAGGGGTCTGGAGGCTGGGATGC  
TCTGCCAGCTCAGAGGATGTTGACTCTGGAGTGGGAGTCCGAAGGACTGCAAACAACAGCGTGGAAGATGAACTGGAGAT  
GGCCACCGTCAGGCATCGGCCGAAGCCCTTGAGCTTCTGGAAGCCCAGAGCAAATTTACCAAGAAAGAGCTTCAGATCC  
TTTACAGAGGATTTAAGAACGAATGCCCCAGTGGTGTGTTAATGAAGAAACCTTCAAAGAGATTTACTCGCAGTTCCTT  
CCACAGGGAGACTCTACAACATATGCACATTTTCTGTTCAATGCATTTTGATACAGACCACAATGGAGCTGTGAGTTTCGA  
GGATTTTCATCAAAGGTCTTTCATTTTGCTCCGGGGACAGTACAAGAAAACTCAATTGGGCATTTAATCTGTATGACA  
TAAATAAAGATGGCTACATCACTAAAGAGGAAATGCTTGATATAATGAAAGCAATATACGATATGATGGGTAATGTACA  
TATCCTGTCTCAAAGAAGATGCTCCAGACAACACGTTGAAACATTTTTTCAGAAAATGGACAAAAATAAAGATGGGGT  
TGTTACCATAGATGAGTTCATTGAAAGCTGCCAAAAAGATGAAACATAATGCGCTCCATGCAGCTCTTGAAAATGTGA  
TTTAACCTGTCAAATAGATCTGAATCCAACAGACAAATGTGAATCTTACCACCTTAAAGTTGGAGCTACCACCTT  
TAGCATAGATTGCTCAGCTTGACACTGAAGCATATATGCAAACAAGCTTTGTTTTAATATAAAGCAATCCCCAAAAGAT  
TTGAGCTTTCAGTTATAAATTTGCATCCTTTTCATAATGCCACTGAGTTCAGGGGATGGTCTAACTCATTTCTACTCTG  
TGAATATTTCAAAGTAATAGAAATCTGGCATATAGTTTATTGGTTCCTTAGCCATGGGATTATTGAGGCTTTCACATATC  
AGTGATTTTTAAATATCAGTGTTTTTGTCTACTCATTGTATGTATTAGTCTTAGGATTTTGAATGGTTTTCTAATATA  
GTGACATCTGCATTTAATTTCCAGAAATTAATTAATTTTCATGTTGAATGCTGTAATTCATTTAAATTCATTTTATA  
TACTTTAAGGAAACAAGATTACAACAATTAATAAACACATAGTTCCAGTTTCTATGGCCTTCCCACCTTCTGTTAGAAA  
TTAGTTTTATCTGGCATTTTAAACATTTAAAAATTATTAACATTTAAAAATTAGTTTATTATCAGATATCAGCATATG  
CCTAATAAACTTATTTTAATAAGCATTTAATTTTCCATAGTATGTTACAGCCAAGGCCTATATAATAATTTTGGATTG  
TTCAATCTTCTTACAGGCTGTTTTCTATTGTATCAATCATTAGTATCAATCATTAAAGTGAAGTTGAAGAAGGCATCAA  
ACAAAACAAGGATGTTTACAGACATATGCAAAGGGTCAGGATATCTATCCTCCAGTATATAGTAATGCTTAATAACAAGT  
AATCCTAACAGCATTAAAGGCCAAATCTGTCTCTTTCCCTTGACTTCCTTACAGCATGTTTATTTATATTACAAGCCAT  
TCAGGGACAAAGAAAGAAACCTTGACTACCCCACTGTCTACTAAGAACAACAGCAAGCAAAATTAGCAAGCAAAATTC  
CTTTGAAAGCACCAAGTGGTTCATTACATTGACAACCTACTACCAAGATTTAGTAGAAAATAAGTGCTCAACAATAATCC  
AGATTACAGTATGATTTAGCTCATCATAATTAGATTATTTTAAATCATCTTAGCCAAAAGTGTAAAGTTGCCACATTAC  
TAAAGCCACACATCGTCCCTGTTTTGTAGAAATATCACAAGACCAAGAGGCTACAGAAGGAGGAAATTTGCAACTGT  
CTTTGCAACAATAAATCAGGTATCTATTCTGGTGTAGAGATAGGATGTTGAAAGCTGCCCTGCTATCACCAGGTAGAAA  
TTAAGAGTAGTACAATACATGTACACTGAAATTTGCCATCACGTGTTTGTGTAACTCAATGTGCACATTTTGTATTTCA  
AAAAGAAAAATAAAAGCAAAATAAAATGTTAAAAA

human kchip4N1S protein

MATVHRHPEALELLEAQSKFTKKELQLLYRGFKNECPSGVVNEETFKEIYSQFFPQGDSTTYAHFLFNAEDTDHNGAVSF  
EDFIKGLSILLRGTVQEKLNWAFNLVDINKDGYITKEEMLDIMKAIYDMMGKCTYPVLKEDAPRQHVETFFQKMDKNKDG  
VVTIDEFIESCQKDNIMRSMQLFENV

FIGURE 56

Human kchip4N1 cDNA (by joining kchip4N1 N + 5'UT and kchip4C, CD: 248-949)  
GTGCGCTGTGGTAGCAGGGGGGAAGCCCCGCCAGCCAAATGCCAGGATCAGCATGAGAAGCTGGACTTTAGCCCAGGTCT  
GTCTCACCCTCGGGGGGGCGCCGGCTTTGCAGGGTGCACTTGCCAGGAGCTGCTCACTTTTTCCCTTGCAAGTCTTTGT  
TCCAAGCCTGACGTTGCTACGATTCTGTAATTAACCTCCCTCCACTCCAAAGGGGTCTGGAGGCTGGGATGCTCTGCCAGC  
TCAGAGGATGTTGACTCTGGAGTGGGAGTCCGAAGGACTGCAACAGTGGGTATTGTTGTGATTATATGTGCATCTCTGA  
AGCTTCTTCATTTGCTGGGACTGATTGATTTTCGGAAGACAGCGTGGAGATGAACTGGAGATGGCCACCGTCAGGCAT  
CGGCCTGAAGCCCTTGAGCTTCTGGAAGCCCAGAGCAAATTTACCAAGAAAGAGCTTCAGATCCTTTACAGAGGATTTAA  
GAATGAATGCCCCAGTGTGTTGTTAATGAAGAAACCTTCAAAGAGATTACTCGCAGTTCTTTCCACAGGGAGACTCTA  
CAACATATGCACATTTTGTTCATGCAATTTGATACAGACCACAATGGAGCTGTGAGTTTCGAGGATTTTCATCAAAGGT  
CTTTCCATTTTGTCTCCGGGGGACAGTACAAGAAAACTCAATGGGCATTTAATCTGTATGACATAAATAAGATGGCTA  
CATCACTAAAGAGGAAATGCTTGATATAATGAAAGCAATATACGATATGATGGGTAAATGTACATATCCTGTCTCTCAAAG  
AAGATGCTCCAGACAACACGTTGAAACATTTTTTCAGAAATGGACAAAAATAAGATGGGGTTGTTACCATAGATGAG  
TTCATTGAAAGCTGCCAAAAGATGAAAACATAATGCGCTCCATGCAGCTCTTTGAAAATGTGATTAACTTGTCAAATA  
GATCCTGAATCCAACAGACAAATGTGAACATTTCTACCACCTTAAAGTTGGAGCTACCACTTTTAGCATAGATTGCTCA  
GCTTGACACTGAAGCATATTATGCAACAAGCTTTGTTTTAATATAAAGCAATCCCCAAAAGATTTGAGCTTTAGTTAT  
AAATTTGCATCCTTTTCATAATGCCACTGAGTTGAGGGGATGGTCTAACTCATTTTCACTCTGTGAATATTCAAAAGTA  
ATAGAATCTGGCATATAGTTTATTGGTTCCTTAGCCATGGGATTATTGAGGCTTTCACATATCAGTGATTTTAAATAT  
CAGTGTTTTTTGTCTACTCATTTGTATGATTTCAGTCCCTAGGATTTTGAATGGTTTTCTAATATAGTGACATGTCATTTA  
ATTTCCAGAAATTAATTAATTTTCATGTTTGAATGCTGTAATTCATTAAATTCATTTATATACTTTAAGGAAACAA  
GATTACAACAATTAACAAAAACATAGTTCAGTTTCTATGGCCTTCCCACCTTCTGTTAGAAATTAGTTTTATCTGGCA  
TTTTTAAACATTTAAATTAATTAACATTTAAATTAAGTTTATTATCAGATATCAGCATATGCCTAATAAACTTATT  
TTAATAAGCATTTAATTTCCATAATATGTTACAGCCAAGGCTATATAATAATTTTGGATTGTTCAATCTTTCTTACA  
GGCTGTTTTTCTATTGTATCAATCATTAGTATCAATCATTAAAGTGAAGTTGAAGAAGGCATCAAACAAAAAAGGATGTT  
TACAGACATATGCAAAGGTCAGGATATCTATCCTCCAGTATATAGTAATGCTTAATAACAAGTAATCCTAACAGCATT  
AAGGCCAAATCTGTCTCTTTCCCTGACTTCCTTACAGCATGTTTATTTATATTACAAGCCATTCAGGGACAAAGAAAG  
AAACCTTGACTACCCCACTGTCTACTAAGAACAACAGCAAGCAAAATTAGCAAGCAAAATTCACTTTGAAAGCACCAGT  
GGTTCATTACATTGACAACTACTACCAAGATTTAGTAGAAAATAAGTGCTCAACAATAATCCAGATTACAGTATGATT  
TAGCTCATCATAATTCAGATTATTTTAAATCATCTTAGCCAAAACGTAAAGTTGCCACATTACTAAAGCCACACACATC  
GTCCCTGTTTTGTAGAAATATCACAAGACCAAGAGGCTACAGAAGGAGGAAATTTGCAACTGTCTTTGCAACAATAAAT  
CAGGTATCTATTCTGGTGTAGAGATAGGATGTTGAAAGCTGCCCTGCTATCACCAGTGTAGAAATTAAGAGTAGTACAAT  
ACATGTACACTGAAATTTGCCATCACGTGTTTGTGTAACTCAATGTGCACATTTTGTATTTCAAAAAGAAAAATAAAA  
GCAAAATAAATGTTAAAAA

Human kchip4N1 protein (by translation of kchip4N1 cDNA)  
MLTLEWESEGLQTVGIVVHCASLKLHLGLIDFSEDSVEDELEMATVHRHPEALELLEAQSFKTKKELQILYRGFKNE  
CPSGVVNEETFKEIYSQFFPQGDSTTYAHFLNFDTHNGAVSFEDFIKGLSILLRGTVQEKLNWAFNLYDINKDGYIT  
KEEMLDIMKAIYDMMGKCTYPVLKEDAPRQHVETFFQKMDKNKDGVTIDEFIESQKDNIMRSMQLFENV

FIGURE 57

Human kchip4N2 cDNA (by joining kchip4N2 N + 5'UT and kchip4C, CD: 90-779)

```
CCTTCTTAAGGAGGTTTAAGGCCTTCCAAAGAAAGCCAGGCAGAGAGGCACTTCTCAGTGGCTGTGGTCGGACCATGACC
TAGCTGACCATGAACTTGGAAGGGCTTGAATGATAGCAGTTCTGATCGTCATTGTGCTTTTTGTAAATTATTGGAACA
GTTTGGGCTGATTGAAGCAGGTTTGAAGACAGCGTGAAGATGAACTGGAGATGGCCACCGTCAGGCATCGGCCTGAAG
CCCTTGAGCTTCTGGAAGCCCAGAGCAAATTTACCAAGAAAGAGCTTCAGATCCTTTACAGAGGATTTAAGAATGAATGC
CCCAGTGGTGTGTTAATGAAGAAACCTTCAAAGAGATTTACTCGCAGTTCTTTCCACAGGGAGACTCTACAACATATGC
ACATTTTCTGTTCAATGCATTTGATACAGACCACAATGGAGCTGTGAGTTTCGAGGATTTTCATCAAAGGTCTTTCCATTT
TGCTCCGGGGACAGTACAAGAAAACTCAATTGGGCATTTAATCTGTATGACATAAAATAAGATGGCTACATCACTAAA
GAGGAAATGCTTGATATAATGAAAGCAATATACGATATGATGGGTAAATGTACATATCCTGTCTCAAAGAGATGCTCC
CAGACAACACGTTGAAACATTTTTTTCAGAAAATGGACAAAAATAAGATGGGGTTGTTACCATAGATGAGTTCATTGAAA
GCTGCCAAAAAGATGAAAACATAATGCGCTCCATGCAGCTCTTTGAAAATGTGATTAACTTGTCAAATAGATCCTGAAT
CCAACAGACAAATGTGAACATACTACCACCCTTAAAGTTGGAGCTACCACCTTTAGCATAGATTGCTCAGCTTGACACT
GAAGCATATTATGCAACAAGCTTTGTTTTAATATAAAGCAATCCCCAAAAGATTTGAGCTTTCAGTTATAAATTTGCAT
CCTTTTCATAATGCCACTGAGTTCAGGGGATGGTCTAACTCATTTTCACTCTGTGAATATTCAAAAGTAATAGAATCTG
GCATATAGTTTTATTGGTTCCTTAGCCATGGGATTATTAGGCTTTTCACATATCAGTGATTTTAAATATCAGTGTTTTT
TGCTACTCATTTGTATGTATTCAGTCTAGGATTTTGAATGGTTTTCTAATATAGTGACATCTGCATTTAATTTCCAGAA
ATTAAATTAATTTTCATGTTTGAATGCTGTAAATCCATTAAATTTCCATTTATATACTTTAAGGAAACAAGATTACAACA
ATTAAAAAACACATAGTTCAGTTTCTATGGCCTTCCCACCTTCTGTAGAAATTAGTTTTATCTGGCATTTTAAACA
TTTAAATTAATTAACATTTAAATTAAGTTTATTATCAGATATCAGCATATGCCAATAAACTTATTTTAATAAGCA
TTTAATTTTCATAATATGTTACAGCCAAGGCCTATATAATAATTTGGATTTGTTCAATCTTTCTTACAGGCTGTTTTC
TATTGTATCAATCATTAGTATCAATCATTAAAGTGGAAGTTGAAGAAGGCATCAACAAAACAAGGATGTTTACAGACATA
TGCAAAGGGTCAGGATATCTATCCTCCAGTATATAGTAATGCTTAATAACAAGTAATCCTAACAGCATTAAAGGCCAAT
CTGTCTCTTTCCCTGACTTCTTACAGCATGTTTATTTATATTACAAGCCATTACAGGGACAAAGAAAGAAACCTTGAC
TACCCCACTGTCTACTAAGAACAACAGCAAGCAAAATTAGCAAGCAAAATTCACTTTGAAAGCACCAGTGGTTCCATTA
CATTGACAACACTACCAAGATTTAGTAGAAATAAGTGCTCAACAACATAATCCAGATTACAGTATGATTTAGCTCATCA
TAATTCAGATTATTTTAATCATCTTAGCCAAAACCTGTAAAGTTGCCACATTACTAAAGCCACACACATCGTCCCTGTTT
GTAGAAATATCACAAAGACCAAGAGGCTACAGAAAGGAGGAAATTTGCACTGTCTTTGCAACAATAAATCAGGTATCTA
TTCTGGTGTAGAGATAGGATGTTGAAAGCTGCCCTGCTATCACCAGTGTAGAAATTAAGAGTAGTACAATACATGTACAC
TGAAATTTGCCATCAGTGTTTGTGTAACTCAATGTGCACATTTTGTATTTCAAAAAGAAAAATAAAGCAAAATAAA
ATGTTAAAAA
```

Human kchip4N2 protein (by translation of hkchip4N2 cDNA)

```
MNLEGLEMI AVLIVIVLFVKLLLEQFGLIEAGLEDSVEDELEMATVRRHPEALELLEAQSKFTKKELQILYRGFKNECPSG
VVNEETFEKIYSQFFPQGDSTTYAHFLFNAFDTDHNGAVSFEDFIKGLSILLRGTVQEKLNWAFNLVDINKDGYITKEEM
LDIMKAIYDMMGKCTYPVLKEDAPRQHVETFFQKMDKNKDGVTITDEFIESCQKDNIMRSMQLFENV I
```

FIGURE 58

Human kchip4N3 cDNA (by joining kchip4N3 N exon1, exon2, and kchip4C, CD:65-817)

GTGGACAGACGCNCCCTGGCGGTGGACTTCTCGAGTCTCGCTTCTGCAACCCTGCGTCCCCAGACATGAATGTGAGGAGGG  
TGGAGAGCATTTTCGGCTCAGCTGGAGGAGGCCAGCTCTACAGGCGGTTTCCTGTACGCTCAGAACAGCACCAAGCGCAGC  
ATTAAAGAGCGGCTCATGAAGCTCTTGCCCTGCTCAGCTGCCAAAACGTCGTCTCCTGCTATTCAAACAGCGTGGAAGA  
TGAAGTGGAGATGGCCACCGTCAGGCATCGGCCTGAAGCCCTTGAGCTTCTGGAAGCCCAGAGCAAATTTACCAAGAAG  
AGCTTCAGATCCTTTACAGAGGATTTAAGAATGAATGCCCAAGTGGTGTGTTAATGAAGAACCTTCAAAGAGATTTAC  
TCGCAGTTCTTTCCACAGGGAGACTCTACAACATATGCACATTTTCTGTTCAATGCATTTGATACAGACCACAATGGAGC  
TGTGAGTTTCGAGGATTTTCATCAAAGGTCTTTCCATTTTGCTCCGGGGGACAGTACAAGAAAACTCAATTGGGCATTTA  
ATCTGTATGACATAAATAAGATGGCTACATCACTAAAGAGGAAATGCTTGATATAATGAAAGCAATATACGATATGATG  
GGTAAATGTACATATCCTGTCTCAAAGAAGATGCTCCAGACAACAGCTTGAAACATTTTTCAGAAAAATGGACAAAA  
TAAAGATGGGGTTGTTACCATAGATGAGTTTCAATGAAAGCTGCCAAAAGATGAAACATAATGCGCTCCATGCAGCTCT  
TTGAAAATGTGATTTAACTTGTCAAATAGATCCTGAATCCAACAGACAAATGTGAATATTCTACCACCTTAAAGTTGG  
AGCTACCCTTTTAGCATAGATTGCTCAGCTTGACACTGAAGCATATTATGCAACAAGCTTTGTTTTAATATAAAGCAA  
TCCCCAAAAGATTTGAGCTTTCAGTTATAAATTTGCATCCTTTTCATAATGCCACTGAGTTCAGGGGATGGTCTAACTCA  
TTTCATACCTGTGTAATATTCAAAGTAATAGAATCTGGCATATAGTTTATTGGTTCCTTAGCCATGGGATTATTGAGG  
CTTTCACATATCAGTGATTTTAAATATCAGTGTTTTTGGCTACTATTGTATGTATTAGTCTAGGATTTTGAATGG  
TTTTCTAATATAGTGACATCTGCATTTAATTTCCAGAAATTAATTAATTTTCATGTTTGAATGCTGTAATCCATTTAA  
ATTCCATTTATATACTTTAAGGAAACAAGATTACAACAATTAATAAACAACATAGTTCAGTTTCTATGGCCTTCCCACC  
TTCTGTTAGAAATTAGTTTTATCTGGCATTTTTAAACATTTAAAAATTATTAACATTTAAAAATTAGTTTATTATCAGA  
TATCAGCATATGCCTAATAAACTTATTTAATAAGCATTAAATTTCCATAATATGTTACAGCCAAGGCCTATATAATA  
ATTTTGATTTGTTCAATCTTTCTACAGGCTGTTTTCTATTGTATCAATCATTAGTATCAATCATTAAAGTGAAGTTGA  
AGAAGGCATCAACAAAACAAGGATGTTTACAGACATATGCAAGGGTCAGGATATCTATCCTCCAGTATATAGTAATGC  
TTAATAACAAGTAATCCTAACAGCATTAAGGCCAAATCTGTCTCTTCCCCTGACTTCCTTACAGCATGTTTATTAT  
ATTACAAGCCATTCAAGGACAAAGAAAGAACCTTGACTACCCACTGTCTACTAAGAACAACAGCAAGCAAAATTAGC  
AAGCAAAATCACTTTGAAAGCACCAAGTGGTTCCATTACATTGACAACTACTACCAAGATTTAGTAGAAAATAAGTGCTC  
AACAATAATCCAGATTACAGTATGATTTAGCTCATATAATTCAGATTATTTTTAATCATCTTAGCCAAAACGTGTAAG  
TTGCCACATTACTAAAGCCACACACATCGTCCCTGTTTTGTAGAAATATCACAAAGACCAAGAGGCTACAGAAGGAGGAA  
ATTTGCAACTGTCTTTGCAACAATAAATCAGGTATCTATTCTGGTGTAGAGATAGGATGTTGAAAGCTGCCCTGCTATCA  
CCAGTGTAGAAATTAAGAGTAGTACAATACATGTACACTGAAATTTGCCATCACGTGTTTGTGTAACTCAATGTGCACA  
TTTTGTATTTCAAAAAGAAAAATAAAGCAAATAAATGTTAAAAA

Human kchip4N3 protein (by translation of kchip4N3 cDNA)

MNVRVESISAQLEEASSTGGFLYAQNSTKRSIKERLMKLLPCSAAKTSSPAIQNSVEDELEMATVRHRPEALELLEAQS  
KFTKKELQLLYRGFKNECPSGVVNEETFKEIYSQFFPQGDSTTYAHFLNFDTHNGAVSFEDFIKGLSILLRGTVQEK  
LNWAFNLYDINKDGYITKEEMLDIMKAIYDMMGKCTYPVLKEDAPRQHVETFFQKMDKNKDGVTIDEFIESCQKDENIM  
RSMQLFENVI

FIGURE 59

rat KChIP4N1x (contig of jTrbal33c02t1 and rkchip4) cDNA (partial), orf: +3,  
coding: 1-821

```
CCCCTCGCCAGACGCCCCCTGGGTCAAGTGGACTCTAAGAGTGTGCTCCAGCATCTATCATCCCTAAAGATGAACGTGAG
AAGGGTGGAAAGCATCTCGGCTCAGCTGGAGGAGGCGAGCTCCACAGGCGGTTTCTCTACCTCAGAACACACCAAGCG
CAGCATTAAAGAGCGGCTCATGAAGCTCTTGCCCTGCTCAGCTGCCAAACGTCGTCTCTGCTATACAAAACAGTGTGG
AAGATGAACTGGAGATGGCCACTGTGAGGCACCGGCTGAAGCCCTGGAGCTGCTGGAGGCCAGAGCAAATTCACCAAG
AAAGAGCTTCAGATCCTTTACAGAGGATTTAAGAATGAATGCCCCAGTGGTGTGTTAATGAAGAAACCTTCAAGGAGAT
TTACTCGCAGTTCTTCCACAGGGAGACTCCACCACATATGCACATTTTCTCTTCAATGCATTGACACGGACCACAATG
GAGCTGTGAGCTTTGAGGATTTATCAAAGGTCTTTCCATTTTGTCTGAGGGACAGTACAAGAAAACTGAATGGGCA
TTTAATTTGTATGACATAAACAAGATGGCTACATCACTAAAGAGGAAATGCTGGACATAATGAAAGCAATTTACGACAT
GATGGGGAATGCACATACCTGTCTCAAGGAAGATGCTCCCCGACAGCAGTGGAGACATTTTCCAGAAGATGGACA
AGAATAAAGATGGTGTGTTACCATAGACGAGTTCATTGAAGTGTCCCAAAAGATGAAAACATAATGCGCTCCATGACAG
CTCTTTGAAAATGTGATCTAGactgtcggtgccttgaccggaggcaaatgtggacgactacacacgagttgaagccacca
tttctagcatagattgtcagctttacactgaggcataattatgcaaacagctttgttttaataataaagacccccgcgccc
aaatttaagttttccagttacaaatccgcactccacgtcactggggtcccgaatgtgctcacttatttcatactctgaga
acactcaaaaggcacagaatctggaacagctttgatccctcagccacgtgttacgggggcttttacagatgagtgatttta
aaacaccagtggttttctacttgtttgtattcagccctggattttaagtggttttctaaaatattttacatctgcatTT
AactTCCAGAAAGCCAATGACCTTTTCAATTTAACTCAATTCATGTAATACTGAAAAAAGGAACAAAGATTATTACAATTA
AAAAAGACCAAAAAACACAGTCCCGATTTCTATAGCTTCTCCACCTGCTGTTAAAGACAGTCATGTATTGGCTTTTTTTT
TTTTTTTAAAAAGAACACTTAAAAAATTAGTTTATTATCAGATGTAGCATATACCTAATAAAAATTATTTTAGTATTTGT
TAATTTTCCATATTCAAGCCAAGGCTCTATATAATCCATGTAACCTTTGGACCTGTTCAATCTTACATGTAGACTGTTTTG
TATTGTGTTCTGAAGTAGAAGTTCAAAGTGTCAAACAACCAAGGATGTTTACAGACTTGCAAAGGGTCCAGATGTCTGT
CCTGCAATGCCTAGTGACGCTTATTAACCAGTAACCTGAAGAGCAGTAACCTGGCAATTCTAGCCACCACCCCTCCCAAG
CCCTTCATGTTCTCACAGCATGTTTATCACACACAAGCCATTGAGGACAGAGAATCCTTGACTGCCCCAAAGCCTACT
AGGAATAAAGATCAAGCAAAATCTTCTTTGAAAACACCAGTGATTCTATCATATTGGAATATACATAAGAGTGTATAGA
AAACGAATGTAGACATTGGACAGTTCATCCGAATTGCATTATGATTAGCACATCATGTAGTTCAAAGGATTCACATTCC
TTTCCGTGATCTTAAGCCAAAACCTGTAGAATTGCCACAACAGTACTAGATATACACACATTCCCTGTTTCGTGGAAATCC
AAGAACCAAGAGGATACGGGAAGAGAAAATTTGCGACTGTCTGCAACAATAAATCAGGTATCTATTCTGGTGTAGAGATA
GGATGTTGAGAGCCGCCCTGCTATCACCAGTGTAGGAATTAAGAGTAGTACAGTACATGTACAGAAATCTGCCATCGCGT
GTTTGTGTAAACTCAATGTGCACATTTTGTATCTCAAAAAGGAAAAATAAAGCAAAATAAAGTGTAAAAAATAAAAAA
AAAAA
```

rat KChIP4N1x (contig of jTrbal33c02t1 and rkchip4) protein (partial)

```
PRQTPLGQVDSKSVAPASIIIPKDEREKGGKHLGSAGGGELHRRFPLPQNNTKRSIKERLMKLLPCSAAKTSSPAIQNSVE
DELEMATVRHRPEALELLEAQSKFKKELQILYRGFKNECPSGVVNEETFKEIYSQFFPQGDSTTYAHFLNFDTDHNG
AVSFEDFIKGLSILLRGTVQEKLNWAFNLVDINKDGYITKEEMLDIMKAIYDMMGKCTYPVLKEDAPRQHVETFFQKMDK
NKDGVVTTIDEFIESQKQDENIMRSMQLFENV
```

FIGURE 60

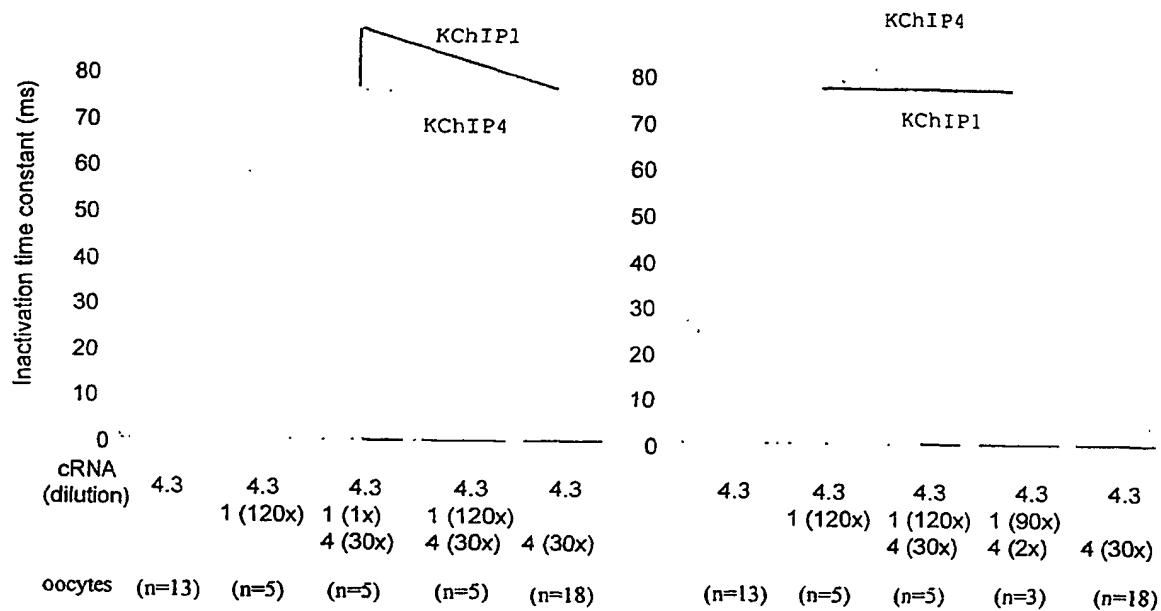


FIGURE 61





## rat KChIP1N (1vn) DNA sequence (CD: 339-1037)

GGCACACAACCCCTGGATTCTTCGGAGAATATGCCGTGAGGTGTTGCCAATTATTAGTTCT  
CTTGCTAGCAGATGTTTAGGGACTGGTTAAGCCTTTGGAGAAATTACCTTAGGAAAACG  
GGGAAATAAAAGCAAAGATTACCATGAATTGCAAGATTACCTAGCAATTGCAAGGTAGG  
AGGAGAGAGGTGGAGGGCGGAGTAGACAGGAGGGAGGGAGAAAGTGAGAGGAAGCTAG  
GCTGGTGGAAATAACCCCTGCACTTGGAACAGCGGCAAAGAAGCGCGATTTTCCAGCTTTA  
AATGCCTGCCCGCGTTCTGCTTGCCTACCCGGAACGGAGATGTTGACCCAGGGCGAGTC  
TGAAGGGCTCCAGACCTTGGGGATAGTAGTGGTCTGTGTTCTCTCTGAAACTACTGCAC  
TACCTCGGGCTGATTGACTTGTTCGGATGACAAGATCGAGGATGATCTGGAGATGACCATG  
GTTTGCCATCGGCCTGAGGGACTGGAGCAGCTTGAGGCACAGACGAACCTTCACCAAGAGA  
GAACTGCAAGTCCTTTACCGGGGATTCAAAAACGAGTGCCCCAGTGGTGTGGTTAACGAA  
GAGACATTCAAGCAGATCTACGCTCAGTTTTTCCCTCATGGAGATGCCAGCACATACGCAC  
ATTACCTCTTCAATGCCTTCGACACCACCCAGACAGGCTCTGTAAAGTTTCGAGGACTTTGT  
GACTGCTCTGTCGATTTTACTGAGAGGAACGGTCCATGAAAACTGAGGTGGACGTTTAA  
TTTGTACGACATCAATAAAGACGGCTACATAAAACAAAGAGGAGATGATGGACATAGTGA  
AAGCCATCTATGACATGATGGGGAAATACACCTATCCTGTGCTCAAAGAGGACACTCCCA  
GGCAGCACGTGGACGTCTTCTTCCAGAAAATGGATAAAAAATAAAGATGGCATTGTAACGT  
TAGACGAATTTCTCGAGTCCTGTCAGGAGGATGACAACATCATGAGGTCTCTACAGCTGTT  
CCAAAATGTCATGTAAGTGAAGGACACTGGCCATCCTGCTCTCAGAGACACTGACAAACAC  
CTCAATGCCCTGATCTGCCCTTGTTCAGTTTTACACATCAACTCTCGGGACAGAAATACC  
TTTTACACTTTGGAAGAATTCTCTGCTGAAGACTTTCTACAAAACCTGGCACCAGTGGCT  
CAGTCTCTGATTGCCAACTCTTCTCCCTCCTCTCTTGAAGGGACGAGCTGAAATCCGA  
AGTTTGTGTTTGAAGCATGCCCATCTCTCCATGCTGCTGCTGCCCTGTGGAAGGCCCTCT  
GCTTGAGCTTAAACAGTAGTGACAGTTTTCTGCGTATACAGATCCGCAACTCACTGCCTC  
TAAGTCAGGCAGACCCTGATCAATCTGAACCAAATGTGCACCATCCTCCGATGGCCTCCC  
AAGCCAATGTGCCTGCTTCTCTCCTCTGGTGGAAGAAAGAACGCTCTACAGAGCACTTA  
GAGCTTACCATGAAAATACTGGGAGAGGCAGCACCTAACACATGTAGAATAGGACTGAAT  
TATTAAGCATGGTGGTATCAGATGATGCAACAGCCCATGTCATTTTTTTTTTCCAGAGGTA  
GGGACTAATAATTCTCCCACTAGCACCTACGATCATAGAACAAGTCTTTTAACACATCC  
AGGAGGGAAACCGCTGCCAGTGGTCTATCCCTTCTCTCCATCCCCTGCTCAAGCCCAGCA  
CTGCATGTCTCTCCCGGAAGGTCCAGAATGCCTGTGAAATGCTGTAACTTTTATACCCTGT  
TATAATCAATAAACAGAACTATTTCTGACAAAAA

## rat KChIP1N (1vn) protein sequence

MLTQGESEGLQTLGIVVVLCSCLKLLHYLGLIDLSDDKIEDDLEMTMVCHRPEGLEQLEAQTN  
FTKRELQVLYRGFKNECPSGVVNEETFQKIYAQFFPHGDASTYAHYLFNAFDTTQTGSVKFED  
FVTALSILLRGTVHEKLRWTFNLVDINKDGYINKEEMMDIVKAIYDMMGKYTYPVLKEDTPR  
QHVDVFFQKMDKNKDGIVTLDEFLESCQEDDNIMRSLQLFQNVN

FIGURE 63

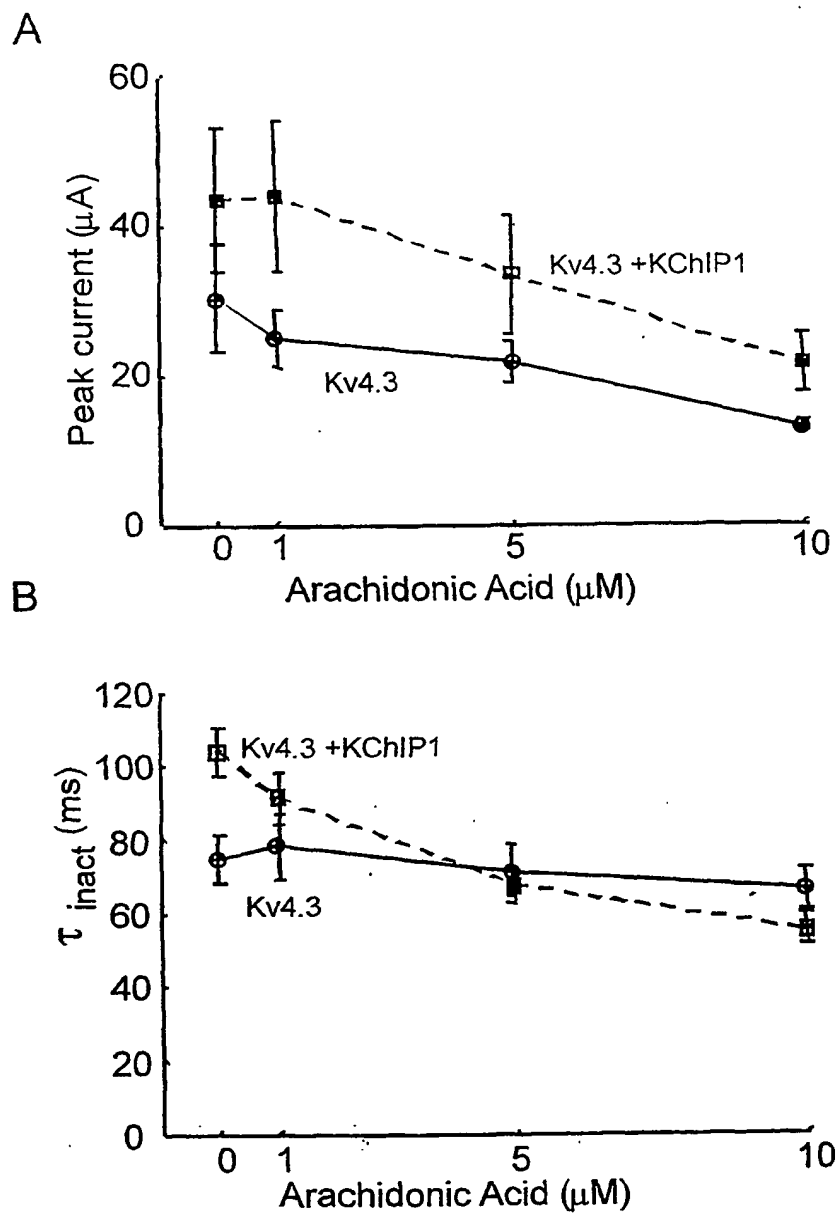


FIGURE 64

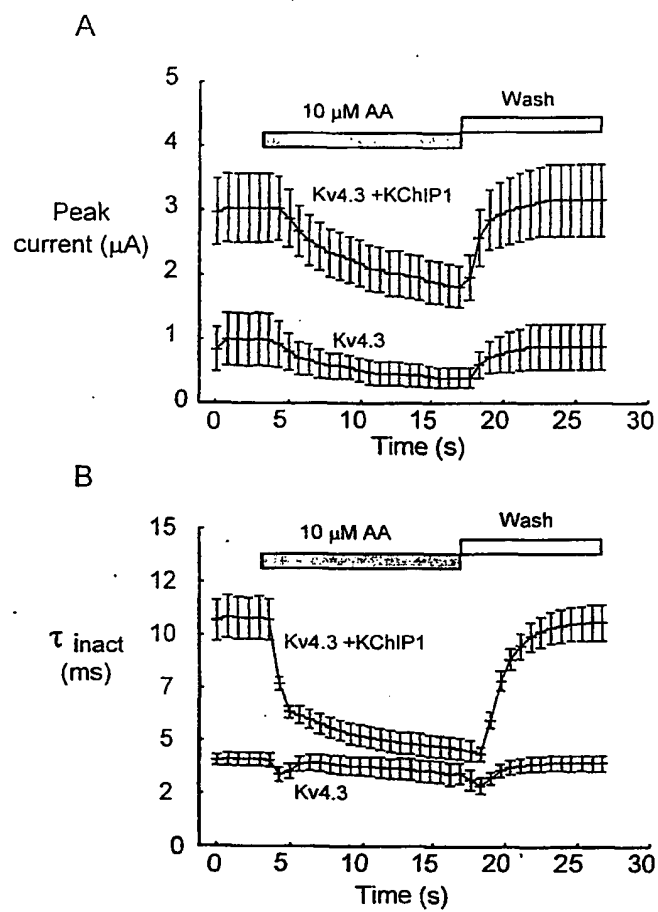
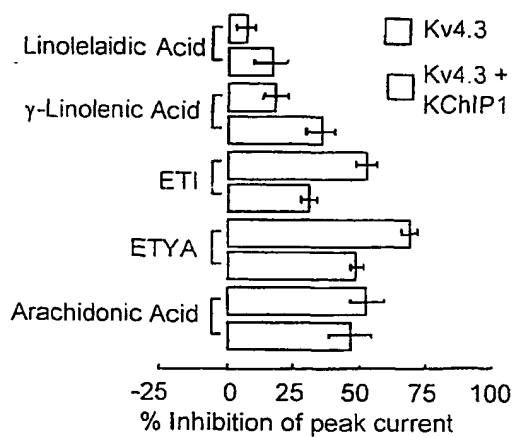


FIGURE 65

A



B

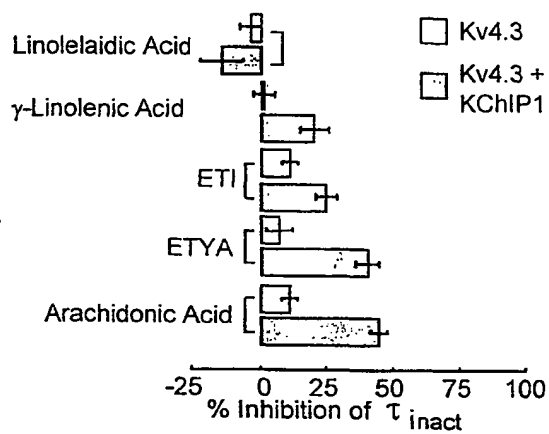
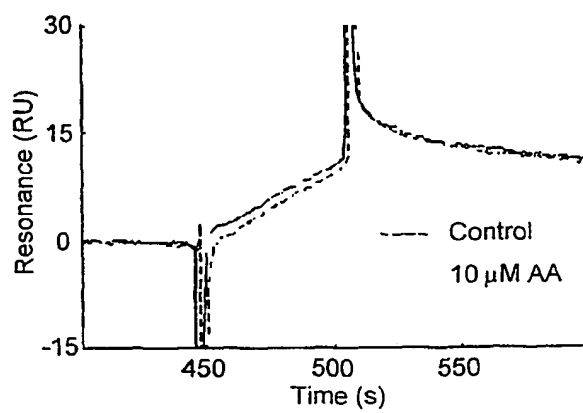


FIGURE 66

A



B

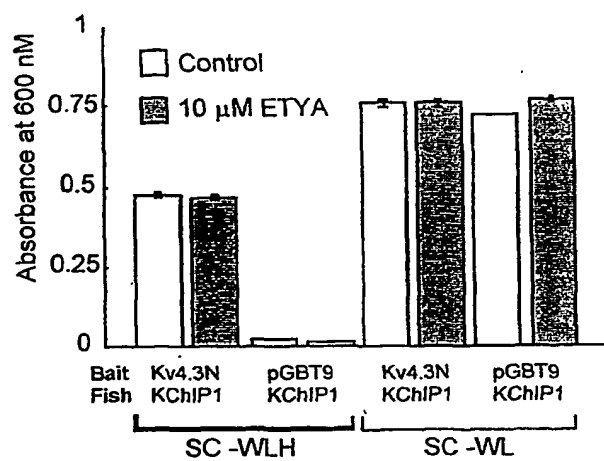


FIGURE 67

rKchip 1N rat phase I

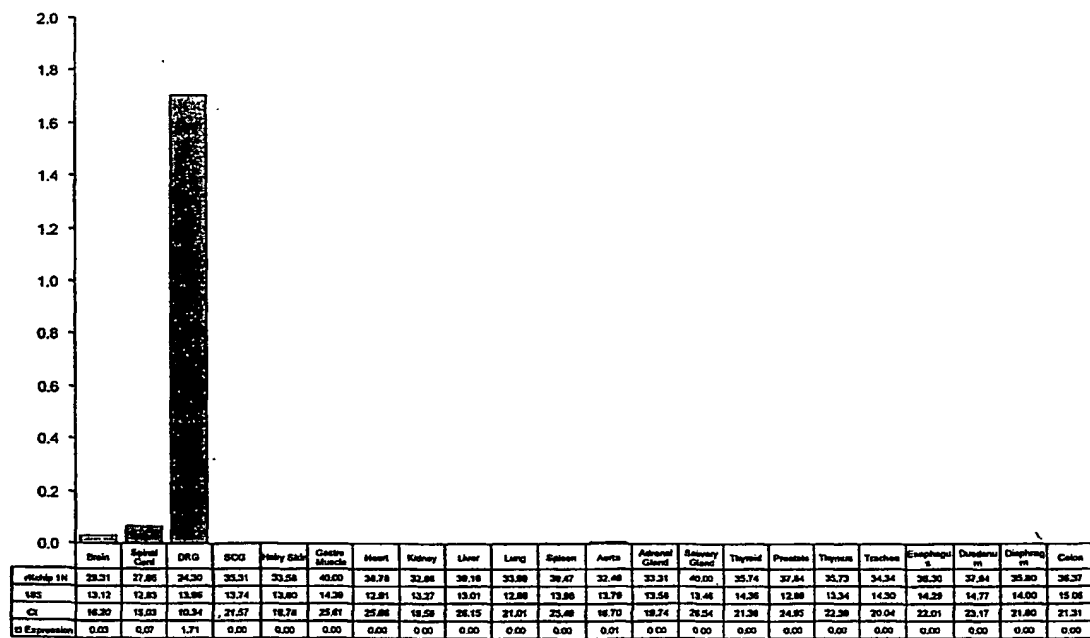


FIGURE 68.

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33/68, A61K 38/17, A01K 67/027

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(71) Applicant: **MILLENNIUM PHARMACEUTICALS INC.** [US/US]; 75 Sidney Street, Cambridge, MA 02139 (US).

(72) Inventors: **RHODES, Kenneth**; 808 Atkinson Circle, Ne-shanic Station, NJ 08853 (US). **BETTY, Maria**; 116 S. Brentwood Drive, Mt. Laurel, NJ 08853 (US). **LING, Huai-Ping**; 17 Wellesley Court, Princeton, NJ 08550 (US). **AN, Wenqian**; 1500 Worcester Road, Apt. #212, Framing-ham, MA 01702 (US).

(74) Agents: **MANDRAGOURAS, Amy, E.**; Lahive & Cock-field, LLP, 28 State Street, Boston, MA 02109 et al. (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**

- with international search report
- with sequence listing part of description published separately in electronic form and available upon request from the International Bureau

(88) Date of publication of the international search report:  
13 March 2003

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: POTASSIUM CHANNEL INTERACTORS AND USES THEREFOR

(57) Abstract: The invention provides isolated nucleic acids molecules, designated PCIP nucleic acid molecules, which encode proteins that bind potassium channels and modulate potassium channel mediated activities. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing PCIP nucleic acid molecules, host cells into which the expression vectors have been introduced, and nonhuman transgenic animals in which a PCIP gene has been introduced or disrupted. The invention still further provides isolated PCIP proteins, fusion proteins, antigenic peptides and anti-PCIP antibodies. Diagnostic methods utilizing compositions of the invention are also provided.

WO 02/026984 A3



## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/30463

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/12 C12N5/10 C07K14/705 C07K16/28 C12Q1/68  
 G01N33/577 G01N33/68 A61K38/17 A01K67/027

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K C12Q G01N A61K A01K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

SEQUENCE SEARCH, EP0-Internal, WPI Data, PAJ, BIOSIS, EMBASE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 31133 A (BETTY MARIA ;AN WENQIAN (US); LING HUAI PING (US); RHODES KENNETH) 2 June 2000 (2000-06-02) the whole document ---	1-54
P,X	WO 00 70049 A (INCYTE GENOMICS INC ;PATTERSON CHANDRA (US); AZIMZAI YALDA (US); Y) 23 November 2000 (2000-11-23) SEQ ID NOS: 15 and 41; (ID 3216587CD1 and 3216587 CB1); claims 1-23 ---	1-54
P,X	WO 01 53312 A (CHEN RUI HONG ;GOODRICH RYLE (US); HYSEQ INC (US); WANG DUNRUI (US) 26 July 2001 (2001-07-26) SEQ ID NOS: 1249, 3035, 4821 and 6607; claims 1-28 --- -/--	1-17

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search

15 August 2002

Date of mailing of the international search report

27. 11. 2002

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HORNIG H.

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/30463

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>AN W FRANK ET AL: "Modulation of A-type potassium channels by a family of calcium sensors."  NATURE (LONDON),  vol. 403, no. 6769,  3 February 2000 (2000-02-03), pages  553-556, XP002209980  ISSN: 0028-0836  the whole document</p> <p>---</p>	1-17
A	<p>BUXBAUM ET AL.: "Calsenilin: a calcium-binding protein that interacts with the presenilins and regulates the levels of a presenilin fragment"  NATURE MEDICINE,  vol. 10, October 1998 (1998-10), pages  1177-1181, XP002132975  cited in the application  the whole document</p> <p>---</p>	
A	<p>CARRION ET AL.: "DREAM is a Ca<sup>2+</sup>-regulated transcriptional repressor"  NATURE,  vol. 398, 4 March 1999 (1999-03-04), pages  80-84, XP000700251  cited in the application  the whole document</p> <p>---</p>	
A	<p>WO 99 49038 A (INCYTE PHARMA INC  ; PATTERSON CHANDRA (US); CORLEY NEIL C  (US); BAN) 30 September 1999 (1999-09-30)  page 29, line 32 -page 48, line 9; claims  1-24; figure 1</p> <p>---</p>	
A	<p>WO 98 16185 A (NPS PHARMA INC)  23 April 1998 (1998-04-23)  the whole document</p> <p>---</p>	
A	<p>VAN HILLE ET AL.: "Identification of two subunit A isoforms of the vacuolar H<sup>+</sup>-ATPase in human osteoclastoma"  THE JOURNAL OF BIOLOGICAL CHEMISTRY,  vol. 268, no. 10, 1993, pages 7075-7080,  XP002132977  abstract; figure 1</p> <p>---</p>	
A	<p>CASTAGNA ET AL.: "Molecular characteristics of mammalian and insect amino acid transporters: implications for amino acid homeostasis"  THE JOURNAL OF EXPERIMENTAL BIOLOGY,  vol. 200, 1997, pages 269-286, XP002132978  the whole document</p> <p>---</p>	

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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/30463

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>LOMBARDI ET AL.: "Structure-activity relationships of the Kvbeta1 inactivation domain and its putative receptor probed using peptide analogs of voltage-gated potassium channel alpha- and beta-subunits"</p> <p>THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 273, no. 46, 13 November 1998 (1998-11-13), pages 30092-30096, XP002132979 the whole document</p> <p>-----</p>	

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

## Continuation of Box I.1

Although claims 42-53 and (21,54)-partially are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Although claim(s) 36-41 (as far as in vivo methods are concerned) are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

## Continuation of Box I.2

Claims Nos.: (21,54)-partially

Present claims 21 and 54 relate to a method of modulating the activity of a PCIP polypeptide or an PCIP-expressing cell, respectively a method to use of a compound to treat a potassium channel associated disorder without giving a true technical characterization. Moreover no such compounds are defined in the application. In consequence, the scope of said claims is ambiguous and vague, and their subject-matter is not sufficiently disclosed and supported (Art. 5 and 6 PCT). No search can be carried out for such purely speculative claims whose wording is, in fact, a mere recitation of the results to be achieved.

Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to antibodies, antisense molecules, ribozymes, triple helix molecules, polypeptides and nucleic acids, the structure of which can be directly derived from SEQ ID Nos. 1 and 2.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1 ; Claims 1-54 partially

A nucleic acid encoding a potassium channel interacting protein (SEQ ID NOS:1 and 2) and subject-matter relating thereto.

Inventions 2-41; Claims: 1-54 partially

Idem as invention 1 but corresponding to SEQ ID NOS: 3-40,48-59,69-72,74-103 and 109 respectively as mentioned in Table I and II. (Invention 2 is limited to SEQ ID NOS: 3 and 4; Invention 3 is limited to SEQ ID NOS: 5 and 6; ....Invention 6 is limited to SEQ ID NOS: 11,12,101 and 102; ....Invention 8 is limited to SEQ ID NOS: 15,16,75 and 76; ....Invention 17 is limited to SEQ ID NOS: 33,34,84 and 85; ....Invention 41 is limited to SEQ ID NOS: 100 and 101);

The Sequence IDs corresponding to the same potassium channel interacting proteins have been put together according to the information provided by the applicant in Table I and II.

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 01/30463

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### Information on patent family members

PCT/US 01/30463

Form PCT/ISA/210 (patent family annex) (July 1992)

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